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Communications

Selective Isolation of Anethole from *Fructus Anisi Stellati* (Star Anise) by Supercritical Fluid Extraction

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Crude anethole over 90% purity (by GC) was obtained by extracting the ground star anise statically (10 min) and then dynamically (30 min) with supercritical carbon dioxide at a density of 0.35 g ml^{-1} and a temperature of 80 °C. Pure anethole (99% by GC) could be obtained by passing the crude anethole onto a column packed with a mixture of silica and activated charcoal and eluted with 2% ethyl acetate in petroleum ether.

Trans-anethole is a naturally occurring food flavouring agent in star anise. The fruit of star anise consists of volatile oil (4–9%) in which *trans*-anethole is the major component (80–90%) while the rest is pinene, safrole and limonene.[1] Typical isolation of anethole from star anise by solvent extraction is tedious, time-consuming, labour-intensive, and causes a considerable contribution to waste production in the laboratory.

In contrast, supercritical fluid extraction (SFE) is a practical alternative for the isolation of anethole because this technique can be performed using non-toxic and non-flammable supercritical CO_2. Although much information has been published to date on SFE,[2–7] the use of SFE to selectively isolate anethole from star anise has not been reported. Heikes[8] has reported the isolation of safrole (an aromatic oil also present in anise) and related alkylbenzenes in sassafras teas by SFE. The isolation of semivolatile flavour compounds from cinnamons using SFE has recently been reported.[9]

Experimental

Standard and Reagents

Trans-anethole standard, analytical-reagent grade methanol, ethyl acetate, petroleum ether, dichloromethane and Celite were obtained from Aldrich (Milwaukee, WI, USA). High- and low-grade carbon dioxide were obtained from Hong Kong Oxygen (Hong Kong).

Instrumentation

A Hewlett-Packard 7680T (Wilmington, DE, USA) supercritical fluid extractor with a Hewlett-Packard 1050 series modifier pump was used for the SFE extractions. All analyses were performed on an HP 5890 gas chromatograph equipped with an HP 5972 mass selective detector fitted with an HP-5 (cross linked 5% phenylmethyl silicone, film thickness 0.25 µm) capillary column (300 × 0.35 mm id). The initial oven temperature was 80 °C; this was ramped to 300 °C at 10 °C min^{-1} and held for 5 min at the final temperature.

Preparation of Samples

Star anise samples were purchased locally and ground to powder with a blender before use. Two layers of filter paper discs cut to the internal diameter of the extraction thimble were placed on the bottom thimble cap and then 0.2 g of Celite was added to the thimble. A mixture of 0.5 g of the sample and 1.5 g of Celite was prepared in a pestle and mortar and then transferred quantitatively to the extraction thimble. Approximately 0.2 g of Celite and two more filter paper discs were placed on top of the sample in the thimble so that the volume of the extraction cell was just filled.

The procedures for the spike extraction experiments were similar to those described above for real samples experiments except that 1.5 g of Celite was spiked with 0.02 g of standard anethole.

SFE Extraction Conditions

A 7 ml extraction thimble (11 mm id × 95 mm) and SFE-grade carbon dioxide (99.90% purity) were used in the extraction. The sample was placed in the thimble as mentioned previously. Before extraction, a modifier was added to the extraction cell by pipetting 0.5 ml of methanol onto the sample in the extraction cell. The thimble was then placed into the extractor at a chamber temperature of 80 °C followed by a 10 min static extraction period and 30 min dynamic extraction with pure supercritical carbon dioxide at a flow rate of 0.5 ml min^{-1}. After the static extraction, the supercritical fluid was depressurized onto a sorbent trap packed with diol and cooled to 5 °C. Once the SFE step was finished, the analytes were recovered by rinsing with 1 ml of dichloromethane at a flow rate of 1 ml min^{-1} and collected in a 1.5 ml vial. The rinse trap and nozzle temperatures were maintained at 30 °C and 25 °C, respectively. The procedure for extracting spiked samples was exactly the same as above except that 0.1 ml of methanol was added in the extraction cell to wet the sample.

Results and Discussion

Selectivity

The selectivity of the method was assessed by extracting real samples using the optimum SFE conditions. Anethole over 90% purity (by GC) was consistently obtained after SFE for 30 min. Under the conditions used, the average amount of anethole extracted was 6.78% and other components were not extracted. Table 1 shows the results of spike recoveries on samples under dry and wet conditions.

Method Validation on Real Samples

Since good spike recoveries do not necessarily indicate good recovery of real samples because the analytes are not necessarily in the same chemical or physical locations in or on the sample matrix, additional validation methods are required to establish the reliability of the proposed SFE method.

The extraction efficiency of the SFE method was performed by comparing the quantities of anethole extracted from real samples using solvent extraction, and SFE for 30 min with supercritical CO_2. The average amount of anethole extracted from 10 replicate real samples by SFE, and solvent extractions were 6.78% (3.2% RSD) and 5.93% (8.2% RSD), respectively. The amount of anethole found in the samples by both methods was within the range quoted in the literature (3–8%).[1] The SFE results correlated with the solvent extraction data for all the real and spiked samples. The relatively low RSD value obtained by the SFE method reflected greater selectivity and precision than the solvent extraction method. It showed that SFE for 30 min with supercritical CO_2 was superior to dichloromethane solvent extraction (12% more extracted anethole than with dichloromethane extraction).

The reliability of the proposed SFE method was assessed by multiple sequential extractions of a single sample using SFE and solvent extractions. Star anise was extracted with dichloromethane (15 h reflux) after SFE for 30 min with supercritical CO_2 to see whether or not any additional anethole was recovered. In contrast, the dichloromethane extract performed after the supercritical CO_2 extraction yielded a minimal amount (about 1%) of additional anethole, indicating that supercritical CO_2 yielded quantitative recovery ($>90\%$) of the native anethole.

Table 1 Recovery of anethole from 10 replicates spiked with 0.02 g anethole

Supercritical fluid	Matrix condition*	Average % recovery†	RSD (%)
CO_2	dry	80	2
CO_2	wet	95	2
CO_2 + 5% methanol	dry	65	3
CO_2 + 5% methanol	wet	71	3
CO_2 + 10% methanol	dry	45	3
CO_2 + 10% methanol	wet	52	3

* Celite 1.5 spiked with 0.02 g anethole. Wet condition means 0.1 ml methanol added to the sample before the extraction. † Experimental conditions: 10 min static followed by 30 min dynamic extraction at 80 °C and 1916 psi.

Conclusion

The developed method is especially suitable for the determination of anethole in food, natural products and/or biological samples. The first fraction collected after the SFE step can be analysed directly by GC or HPLC without further purification. The optimized SFE method is fast, efficient and selective as compared with the solvent extraction method. It consumes far less organic solvents, by an order of 10 to 100 times, than solvent extraction. This substantially reduces the cost per sample, both for purchase of solvents as well as the disposal of the used toxic wastes. SFE is a significantly more 'environmentally friendly' method than traditional methods of sample preparation.

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