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Identification of volatile organic compounds of *Anethum L* juice using headspace and direct solid phase micro extraction (HS-SPME, DI-SPME) gas chromatography mass spectrometry(GC/MS)

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Abstract

A comparison of headspace and direct immersion solid-phase micro extraction (HS-SPME and DI-SPME) coupled to gas chromatography (GC) with mass spectrometry detection for the analysis of volatile organic compounds of *Anethum L* juice. The major components were: Carvone (61.63-70.25%), Dihydrocarvone (14.51-21.25%), Dillapiole (4.34-18.42%), Carvacrol (0.81-2.24%), Estragole (0.30-0.62%), Fenchone (0.35-0.51%) and Myrtenal (0.34-0.51%). The proposed methods were successfully applied to determining the fruit juices.

Kew words: *Anethum L*, HS-SPME, DI-SPME, GC/MS, Carvone.

Introduction

Anethum graveolens L. (dill) is a sparse looking plant with feathery leaves and tiny yellow flowers. Some pharmacological effects have been reported, such as antimicrobial [1,2], antihyperlipidaemic and antihypercholesterolaemic [3] activities. As a folk remedy, dill is considered for some gastrointestinal ailments such as flatulence, indigestion, stomachache and colic [4]. Dill fruit has an antispasmodic effect on the smooth muscles of the gastrointestinal tract [5].

Analyses of volatile or semi-volatile organic environmental pollutants, flavor or fragrance components, and many other samples usually begin with concentrating the analytes.

However, due to the increasing requirements of environmental and toxicological regulations, the current detection limits cannot meet all needs, so sample enrichment is frequently required before introduction into the chromatographic system. As a result, sample preparation is most time consuming and costly part of many analyses. The goal of any sample preparation step is to yield the analytes of interest in a form and concentration that can be readily analyzed. Although various extraction methods using highly efficient instruments such as solid phase extraction (SPE) [6-10], column preconcentration [11-12], ultrasonic, soxhlet extraction and sonication [13-14], supercritical fluid extraction (SFE) [15-16] and liquid liquid extraction (LLE) [17-18] have been used for the preconcentration. The choice of appropriate sample preparation method, greatly influences the reliable and accurate analysis. Some of the techniques given above such as LLE, SFE and SPE are being tedious and time consuming. A large volume of solvent and sample is required for these techniques which are expensive, health hazard and harmful to environment. It is important to concentrate on a rapid, sensitive, solvent free, less laborious and economical technique. Solid phase micro extraction (SPME) has been proposed as a promising alternative for the sampling, isolation, enrichment of analyte.

Results and Discussion

The *Anethum L.* juice (the local name Ab Shevid) was prepared from a supermarket of Mashhad town, Khorasan Razavi province of Iran. The sample is placed in a vial, which is sealed with septum type cap. The fiber should be cleaned before analyzing of each sample to prevent high background in chromatogram due to contamination. The SPME needle is used to pierce the septum and fiber is withdrawn from the needle of SPME sampling device and exposed to sample. Depending upon the matrix and analyte of interest, there are two modes of extraction: headspace and direct immersion sampling mode. Magnetic stirring is widely used for agitation in both HS-SPME and DI-SPME. It accelerates the transfer of analytes from the sample matrix to coating of fiber.

Table 1. **The results of chemical components of *Anethum L.* juice using HS-SPME and DI-SPME**

Retention time	Compound	*SI%	Head Space(cont.%)	Direct Immersed(cont.%)
3.047	*β*- Cymen	80	0.04	0.01
4.304	Fenchone	93	0.51	0.35
5.757	Myrtenal	70	0.51	0.34
7.057	Dill ether	80	0.24	0.17
7.520	Dihydrocarvone	97	21.25	14.51
9.638	Carvone	97	70.25	61.63
10.742	Estragole	92	0.62	0.30
11.354	Carvacrol	95	0.81	2.24
20.093	Myristcin	85	0.42	1.24
24.256	Dillapiole	93	4.34	18.42
Total			98.99	99.21

*Similarity Index using with the NIST and Wiley libraries

The analysis of the chemical components by the both technique were performed by GC/MS. Figs. 1 and 2, show the chromatograms of HS-SPME and DI-SPME of *Anethum L.* juice, respectively. The compounds were identified by comparing the obtained mass spectra of the analytes with those of authentic standards from the NIST and Wiley libraries with a resemblance percentage above 85% .The calculated retention index (RI) for each component of the analytes was compared with literatures .In the present work extraction of *Anethum L.* juice components using headspace and direct immersed solid phase micro extraction (SPME) were done. Table1,shows the common components of *Anethum L.* juice using HS-SPME and DI-SPME. (Comparison in Figure 3 proves that the percentage of components obtained in two ways nearly the same, and the major constituent is Carvone in the both HS-SPME(70.25) and DI-SPME(61.63) methods.

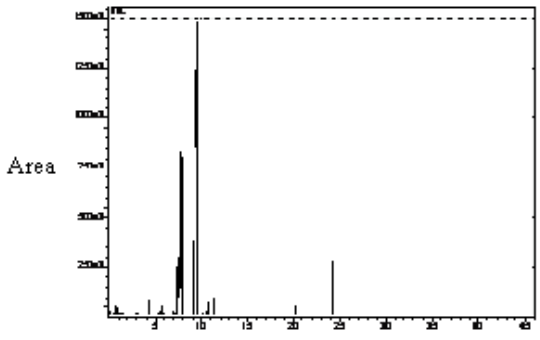

Figure1. Chromatogram of *Anethum L.*juice using HS-SPME.

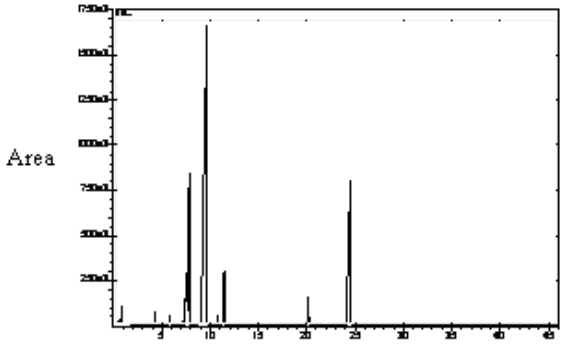

Figure2. Chromatogram of *Anethum L.* juice using DI-SPME.

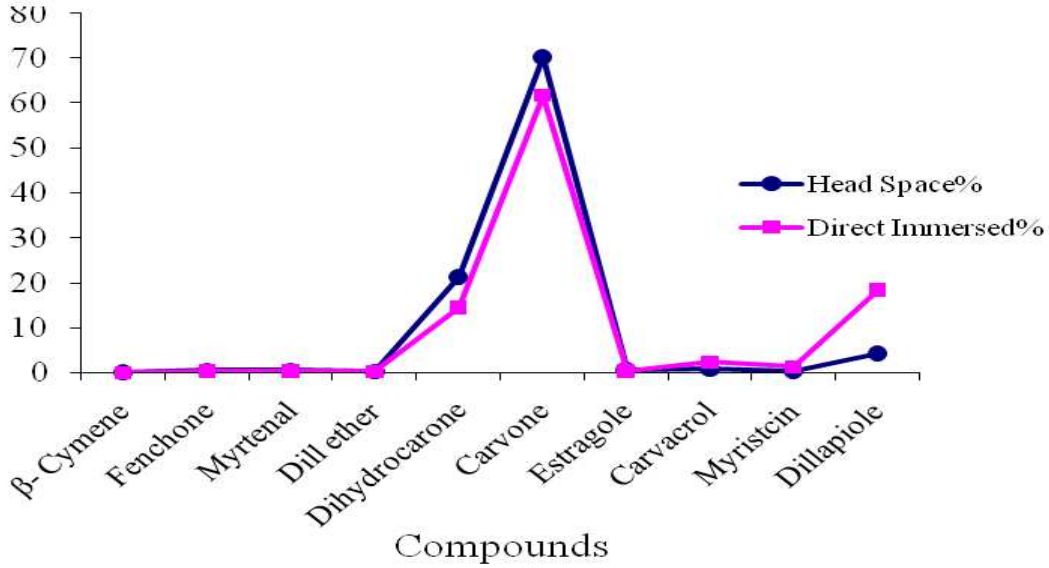

Figure3. Chemical components of *Anethum L.* juice using HS-SPME and DI-SPME.

Materials and Methods

Plant material

The *Anethum L.* juice (the local name Ab Shevid) was prepared from a usual supermarket of Mashhad town , Khorasan Razavi province of Iran.

Instrumentation and Conditions

Instruments:	Chromatographic separation was performed on Shimadzu GC/MS model QP 5050 .
Column:	BD-5 (30 × 0.02mm, film thickness 0.32μm).
Injector:	Injection (Injector) with adjustable temperature programs - SPME fiber injection are in the gap starting column temperature 60°C of a minute in this temperature was kept until the temperature 200°C speed 3°C/ min was heat.
Carrier gas:	helium, constant flow mode, 1.7 mL/min
Fiber:	Fiber coated with Poly (dimethyl silioxane)-di vinyl benzene (PDMS-DVB) from Supelco Company
SPME conditions:	The fiber is exposed in the vapor phase above the gaseous, liquid and solid phase or immersion of fiber coating directly into the liquid sample. The extraction time(10 min) the fiber was with drawn into the needle, then the needle was removed from the septum and inserted directly onto the injection port of the GC. The desorption of analytes from the fiber coating was performed by heating the fiber in the injection port at 260˚C for (5 min).
Samples:	Sampling is based on the transfer/adsorption of analytes to thin film of stationary polymeric phase coated on SPME fiber. Adsorption of analytes depends upon its partition between sample and stationary phase.5ml of Anethum L. juice was transferred in to a 15ml glass vial.

Working of SPME

It was invented in 1987–1989 [19-23]. It comprised of a holder assembly with a thin fused silica fiber coated with a sorbent. The SPME holder assembly provides protection to fiber and allows piercing of rubber septum. The fiber should be cleaned before analyzing any sample as the contaminants are responsible for the background in chromatogram. It is done in desorption chamber of GC by running gas. During the process, the fiber is lowered into the vial which is sealed with a septum type cap. The fiber is extended into the sample through needle. It results in the adsorption of analyte on the fiber. After sampling, the fiber is retraced within its holder for protection. The analytes are desorbed from the fiber using the mobile phase. This requires a special interface which consists of six port injection valve and a desorption chamber. Desorption chamber is placed in the position of injection loop. When sample is extracted, the fiber is inserted into the desorption chamber at the ‘load’ position. After changing the injector to ‘inject’ position, the mobile phase comes in contact with the fiber. Desorption of analytes occur and mobile phase delivers them to the GC column where they get separated and detected by suitable detector.

SPME type
SPME needle is used to pierce the septum and fiber is withdrawn from the needle of SPME sampling device and exposed to sample. Depending upon the matrix and analyte of interest, there are two modes of extraction: headspace and direct immersion sampling mode.

Headspace mode
In this mode, the fiber is exposed in the vapor phase above the gaseous, liquid and solid phase. In this case, only volatile analytes can be sampled. It protects damage of fiber coating from high molecular weight and other non-volatile contaminants present in sample matrix, as the fiber is not in direct contact with the sample. Analytes are adsorbed on fiber coating by crossing the air barrier present between extraction phase and sample surface.

Direct immersion mode
DI involves the immersion of fiber coating directly into the liquid sample. The analytes are adsorbed directly on the fiber coating as it is in direct contact with sample matrix. These analytes exhibit high affinity for aqueous solutions and are less volatile due to their polar nature. Therefore, vapor pressure is low and it requires higher temperature for sorption by HS-SPME. In such cases, a loss of information may occur due to the decomposition of analytes at very high temperature used for HS-SPME.

Conclusion

In recent years, this novel method has been widely adopted in many fields, including analyses of plant materials, environment, food, water and drugs [24-33]. Indeed, its use has been extended to the analyses of a great variety of matrices (gas, liquid and solid). In this study, HS-SPME is used based on the equilibrium of analytes among the three system phases: the coated fiber, the headspace and the sample solution. The limiting step in this extraction is the diffusion of the analytes through the system [34].

The chromatograms of HS-SPME and DI-SPME are shown in Figs. 1 and 2, the results are nearly the same for the both method. But the cont.% for the chemical compositions in some cases are different. These differences refer to the type of the analysis. As it is shown in Table1, in all the cases there are 10 compounds for the methods. It means both HS-SPME and DI-SPME as the rapid techniques, can use for the analysis of plants like *Anethum L.* juice instead of the time consuming methods [35-37] .

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