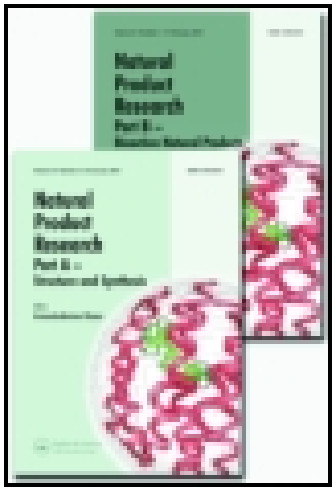

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Rapid monitoring of carvacrol in plants and herbal medicines using matrix solid-phase dispersion and gas chromatography flame ionisation detector

Marzieh Rashidipour[a], Rouhollah Heydari[b], Alireza Feizbakhsh[c] & Payman Hashemi[d]

[a] Young Researchers and Elite Club, Khorramabad Branch, Islamic Azad University, Khorramabad, Iran

[b] Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences, P.O. Box 68149-89468Khorramabad, Iran

[c] Department of Chemistry, Islamic Azad University, Central Tehran Branch (IAUCTB), P.O. Box 14676-86831Tehran, Iran

[d] Department of Chemistry, Lorestan University, Khorramabad, Iran

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Rapid monitoring of carvacrol in plants and herbal medicines using matrix solid-phase dispersion and gas chromatography flame ionisation detector

Marzieh Rashidipour[a], Rouhollah Heydari[b]*, Alireza Feizbakhsh[c] and Payman Hashemi[d]

[a]*Young Researchers and Elite Club, Khorramabad Branch, Islamic Azad University, Khorramabad, Iran;* [b]*Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences, P.O. Box 68149-89468 Khorramabad, Iran;* [c]*Department of Chemistry, Islamic Azad University, Central Tehran Branch (IAUCTB), P.O. Box 14676-86831 Tehran, Iran;* [d]*Department of Chemistry, Lorestan University, Khorramabad, Iran*

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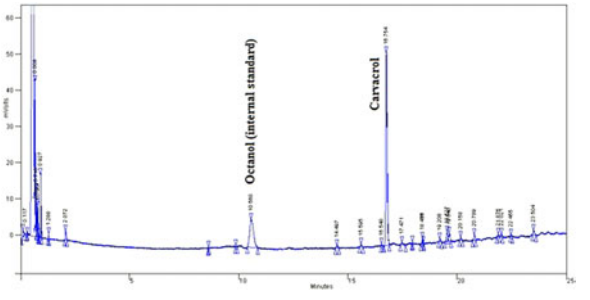

Matrix solid-phase dispersion (MSPD) method coupled with gas chromatography flame ionisation detector as a quick and easy extraction technique has been developed to extract carvacrol from plants and herbal medicines. Influence of important parameters on the MSPD method efficiency, such as the sorbent material, the ratio of sample to sorbent material, elution solvent and volume of the elution solvent has been evaluated and optimised. Carvacrol was successfully extracted by diatomaceous earth as sorbent with 350 μL of dichloromethane as elution solvent. The calibration curve showed good linearity ($r^2 = 0.9965$) and precision (RSD $< 8.16\%$) in the concentration range of 0.5–100 μg mL^{-1} for carvacrol. The limit of detection and limit of quantification were 0.1 and 0.5 μg mL^{-1}, respectively. The recoveries were in the range of 74.4–80.5% with relative standard deviation (RSD) values ranging from 8.4% to 9.8%. The reported MSPD extraction method revealed to be simpler and faster than conventional methods used to quantify carvacrol from plants and herbal medicines.

Keywords: carvacrol; matrix solid-phase dispersion; herbal medicines; GC-FID

1. Introduction

Carvacrol is a phenolic compound that has been found in a number of plants and herbal medicines (De Vincenzi et al. 2004). Essential oils containing carvacrol exhibit effective antimicrobial and antioxidant properties that make them suitable as food preservatives (Burt 2004; Mastelic et al. 2008). For this reason, in recent years researchers have devoted

*Corresponding author. Email: rouhollahheydari@yahoo.com

considerable attention on essential oils and their applications (Rahimi-Nasrabadi et al. 2012; Belaqziz et al. 2013; Jemia et al. 2013; Hassine et al. 2014; Xiea et al. 2014).

A number of experimental procedures for determination of carvacrol from plants have been reported. In most of these methods carvacrol as a component of the essential oil is extracted by hydrodistillation (HD) and analysed by gas chromatography-mass spectrometry (GC-MS) (Sefidkon & Jamzad 2005; Sarrazin et al. 2012; Ghasemi Pirbalouti et al. 2013; Jamali et al. 2013; Rowshan et al. 2013). Several methods including reversed-phase high-performance liquid chromatography (RP-HPLC) using UV-vis (Alekseeva 2009; Hajimehdipoor et al. 2010; Hadad et al. 2014), fluorimetric (Viñas et al. 2006), electrochemical detection (Ternes et al. 1995) and hydrodistillation-headspace solvent microextraction (HD-HSME) with gas chromatography flame ionisation detector (GC-FID; Kiyanpour et al. 2009) were applied for determination of carvacrol in the presence of thymol and other phenolic compounds. Sample preparation in most of these methods is performed by using HD and solid-phase extraction (SPE) which are time consuming and expensive. Therefore, developing an inexpensive and fast analytical method for carvacrol monitoring in plants and herbal medicines is essential. To the best of our knowledge, this study is the first report describing determination of carvacrol in herbal medicines preparations.

HD is one of the oldest methods and most widely used approaches for conventional extraction of volatile compounds from solid samples. It is the most conventional of all methods and consists of a simple distillation process. However, the HD process is usually time consuming and requires large amounts of sample. Therefore, the development of simple and effective extraction methods is of great interest in recent years.

Matrix solid-phase dispersion (MSPD) was developed in 1989 (Barker et al. 1989) and applied to the extraction of a wide range of drugs, pesticides, naturally occurring constituents, essential oils and other compounds from a wide variety of complex samples (Capriotti et al. 2010; Dawidowicz et al. 2011; Yin et al. 2013). The advantages of MSPD extraction are that besides requiring only small amounts of sample and solvents, it is rapid, inexpensive and can be carried out under mild extraction conditions (room temperature and atmospheric pressure).

In the MSPD process, a sample (liquid, semi-solid or solid) is placed in a glass or agate mortar containing an appropriate bonded-phase (C_8, C_{18}, CN, NH_2 and phenyl) or other underivatised sorbent material such as silica gel, florisil, sand, alumina and diatomaceous earth (DE). The mechanical shearing forces produced by the grinding process disrupt the structure of the sample tissue, dispersing the sample over the surface of the support sorbent by hydrophilic and hydrophobic interactions. The process causes the mixture to become semi-dry and free-flowing, and a homogeneous blend of sample and sorbent is resulted. This blend is then packed into a pre-fritted SPE column, and elution of interference compounds and analytes of interest can then take place using suitable solvents. However, no reports about the use of the MSPD method for extraction of carvacrol have been found in the literature.

The objective of this study was to develop an MSPD method for the sample preparation and quantitative extraction of carvacrol from plants and herbal medicines followed by GC-FID determination.

2. Results and discussion

2.1. *Effect of sorbent type*

In the MSPD procedure, the dispersing sorbent is used as not only an adsorption separation material but also a blending solid support to disrupt and disperse the sample. In this study, three sorbents including silica gel, C_{18} and DE were tested. As shown in Figure S1, among the three dispersing adsorbents that were examined, DE obtained highest peak area for carvacrol. Thus, DE was used as the sorbent for further experiments.

2.2. *Effect of elution solvent and its volume*

Polarity of the elution solvent is another important parameter in the MSPD extraction. In this work, various solvents with different polarities, including ethyl acetate, dichloromethane, methanol, ethanol and isopropyl alcohol, were used as elution solvents in the MSPD extraction method. Finally, dichloromethane was selected as elution solvent, because it shows higher extraction efficiency than other solvents (Figure S2). Various experiments were performed by using different volumes of dichloromethane in the range of 350–950 μL. In higher elution solvent volumes (>350 μL), carvacrol signal due to dilution was decreased. As shown in Figure S3, maximum extraction efficiency was obtained using 350 μL of dichloromethane.

2.3. *Effect of dispersing solvent and its volume*

It is very important that the minimum amount of dispersing solvent was added to the sample and sorbent mixture in order to increase contact between the analyte and sorbent. Therefore, extraction was carried out in the presence of various solvents. The results (Figure S4) indicated that the extraction efficiency of carvacrol increased when using 1,4-dioxan as dispersing solvent. Various experiments were performed by using different volumes of 1,4-dioxan in the range of 50–200 μL. As shown in Figure S5, maximum extraction efficiency was obtained by using 100 μL of 1,4-dioxan.

2.4. *Effect of sample mass to sorbent ratio*

A suitable mass ratio of sample to sorbent could allow complete adsorption of the analyte or analytes on the sorbent. In this work, the DE was used as sorbent and dichloromethane as elution solvent. Different ratios of sample mass to DE: 1:1, 1:2, 1:4 and 1:8 were studied. The results in Figure S6 show that the mass ratio of 1:2 has maximum significant effect on the extraction yield of carvacrol. Thus, the ratio of 1:2 was selected for further investigations.

2.5. *Method evaluation*

2.5.1 *Linearity*

Linearity is the ability of the test method to provide results that are directly proportional to analyte concentration within a given range. The linearity of the MSPD method was determined using standard solutions treated with the same method developed for the samples. The calibration curve ($y = 0.0749x + 0.0678$) for carvacrol over the concentration range 0.5–100 μg mL^{-1} exhibited good linearity ($r^2 = 0.9965$).

2.5.2 *Limit of detection and limit of quantification*

The limit of detection (LOD) was defined by the lowest detectable concentration yielding a signal-to-noise ratio (S/N) = 3. On the other hand, limit of quantification (LOQ) was defined as concentration with S/N = 10. LOD and LOQ values were 0.1 and 0.5 μg mL^{-1}, respectively.

2.5.3 *Precision*

To study the intraday precision, five plant samples (*Satureja khuzestanica*, 0.01 g) were exposed to sample preparation and subjected to the optimised method in triplicate on the same day. The relative standard deviation (RSD) value obtained was 8.16 %. To study the interday precision of the method, three same samples were subjected to the optimised method in three different days in triplicate. The RSD value obtained was 10.51%.

2.5.4 *Recovery*

In order to investigate the presence of matrix effects on the proposed method, a recovery study was carried out. Average recoveries and RSD values of the method were evaluated using spiked samples containing carvacrol in different concentration levels. The results shown in Table 1 indicate that the MSPD method gives acceptable accuracy and recovery.

2.5.5 *Specificity*

The specificity of the proposed MSPD procedure was studied by analysing blank extract. The absence of background peaks, above the S/N of 3, at the retention time of the carvacrol, showed that no interferences occurred (Figure S7).

2.6. *Application of MSPD method for analysis of real samples*

In order to evaluate the performance of optimised MSPD, carvacrol extraction from real samples was performed by the MSPD method. The sample extracts were analysed by GC-FID. The results are listed in Table 2. Figure S8 shows a typical GC-FID chromatogram of the *S. khuzestanica* extract by the MSPD method.

The analytical parameters of the proposed method were compared with several reported methods in the literature (Table 3). The results show that LOD and LOQ of carvacrol were improved by using the MSPD–GC-FID. The proposed method can be surely used to monitor and determine carvacrol in plants and herbal medicines.

3. Experimental

3.1. *Chemicals*

Carvacrol was purchased from Indofine Chemical Company (Hillsborough, CA, USA). Methanol, ethanol, dichloromethane, ethyl acetate, isopropyl alcohol, octanol and 1,4-dioxan were purchased from Merck Chemical Co. (Darmstadt, Germany).

Table 1. Average of recoveries and RSD values obtained with the MSPD method for spiked samples ($n = 5$).

Spiked concentration ($\mu g\,mL^{-1}$)	Recovery (%)	RSD (%)
10	74.4	9.8
50	75.8	8.5
100	80.5	8.4

Table 2. The results obtained for various samples by the MSPD method.

Sample	Carvacrol (mg/g)
Dentol	0.06
Dentafort	0.08
Saturex	0.20
S. khuzestanica	0.63
S. reshingeri	0.36
T. vulgaris	0.05

Table 3. The results of analytical performance for several reported methods and the proposed method.

Method	Matrix	LOD ($\mu g\,mL^{-1}$)	LOQ ($\mu g\,mL^{-1}$)	RSD (%)	Recovery (%)	Analysis time (min)[a]	References
RP-HPLC-FLD[b]	Honey	0.0015	0.0052	2.5	96.0–98.0	≈30	Viñas et al. (2006)
RP-HPLC-UV	Plant	0.6	1.8	4.7	97.0	–	Hajimehdipoor et al. (2010)
HD-HSME–GC-FID[c]	Plant	0.23	0.77	9.43	95.0–116.0	≈40	Kiyanpour et al. (2009)
MSPD-GC-FID	Plant and herbal medicines	0.1	0.5	10.51	74.4–80.5	≈30	PM[d]

[a] Analysis time includes sample preparation and chromatographic run time.
[b] Reversed-phase high-performance liquid chromatography-fluorimetric detector.
[c] Hydrodistillation-headspace solvent microextraction–gas chromatography-flame ionizsation detector.
[d] Proposed method.

DE (95% SiO_2) was from Aldrich Chemical Co. (Milwaukee, WI, USA). Octadecyl-functionalised silica gel (C_{18}) and silica gel 60 (15–40 μm) were from Merck Chemical Co.

3.2. *Samples*

Dentol (dental anaesthetic and antiseptic) drop and Saturex capsules (dietary supplement) were obtained from Khorraman Company (Khorramabad, Iran). Dentafort (toothache pain relief) gel was obtained from Ahuradarou Herbal Products Company (Shiraz, Iran). The aerial parts of *S. khuzestanica*, *Satureja reshingeri* and *Thymus vulgaris* were collected from Khorramabad, Iran. Voucher specimens of *S. khuzestanica*, *S. reshingeri* and *T. vulgaris* bearing numbers 4661, 12227 and 6221, respectively, have been lodged at Lorestan Agricultural Research Center Herbarium. Before the extraction, the aerial parts were dried, milled, homogenised and kept at 4°C until analysis. *S. khuzestanica* was used as model sample in the whole optimisation process.

3.3. *Standard solutions preparation*

A stock standard solution (1000 $\mu g\,mL^{-1}$) was prepared by dissolving carvacrol in 1,4-dioxan. Working standard solutions at different concentrations (0.5, 1, 5, 10, 20, 40, 60, 80 and 100 $\mu g\,mL^{-1}$) were prepared by diluting the suitable volume of the stock standard with 1,4-dioxan. Octanol as internal standard (10 $\mu g\,mL^{-1}$) was added to working standard solutions. Internal standard calibration can be used to compensate for variation in analyte recovery and absolute peak areas due to matrix effects and GC injection variability. Prior to the extraction, a known quantity of a known additional analyte is added to each sample and standard.

3.4. *Chromatographic conditions*

The injection was performed using a 10 μL Hamilton gas-tight syringe Model 1701N. The GC system (Varian CP 3800, Middleburg, the Netherlands) equipped with a CP-SIL 8CB-MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) was used. Nitrogen was used as the carrier gas at the constant flow rate of 1.0 $mL\,min^{-1}$. The split ratio was adjusted at 1/50. The oven temperature was initially set at 40°C for 12 min, then it was raised to 180°C at a rate of 10°C/min and left constant for 4 min. The injector and FID temperatures were kept at 250°C and 280°C, respectively. Hydrogen and air flow rates were 30 $mL\,min^{-1}$ and 300 $mL\,min^{-1}$, respectively.

3.5. *MSPD procedure*

A 0.01 g of sample was weighted and then blended thoroughly with 0.02 g of DE and 100 μL of 1,4-dioxan containing 10 $\mu g\,mL^{-1}$ octanol as internal standard in an agate mortar for 5 min using an agate pestle to obtain a homogeneous mixture. The mixture was quantitatively transferred to an empty syringe containing a polyethylene frit at the bottom. A second frit was placed on the top of the sample and was slightly compressed with a syringe plunger to remove air and avoid preferential channels. The packed syringe was attached to a vacuum pump. Carvacrol was eluted from the syringe with 350 μL of dichloromethane. Finally, 1 μL of this solution was injected into the GC injector for analysis. Three replicates were performed for each set of experiments during the method optimisation and for real samples analysis.

For construction of the calibration curve, 100 μL of working standard solutions was mixed with 0.02 g of DE and subsequent steps were performed similar to sample preparation according to the MSPD procedure.

4. Conclusion

In this study for the first time, MSPD as a sample preparation method was used to extract carvacrol from plants and herbal medicines. Monitoring of carvacrol was performed using less amount of samples and less sample preparation time when compared with HD method. The results of this study showed that the proposed MSPD method followed by GC is a useful tool for fast monitoring of carvacrol in plants and herbal medicines.

Supplementary material

Supplementary material relating to this paper is available online, alongside Figures S1–S8.

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