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Microwave-assisted phase-transfer catalysis for the rapid one-pot methylation and gas chromatographic determination of phenolics

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ABSTRACT

Microwave-assisted phase-transfer catalysis (PTC) is reported for the first time, for the one-step extraction–derivatization–preconcentration and gas chromatographic determination of twenty phenols and ten phenolic acids. The well established phase-transfer catalytic methylation is largely accelerated when heating is replaced with the "greener" microwave irradiation. The overall procedure was thoroughly optimized and the analytes were determined by GC/MS. The method presented adequate analytical characteristics being more sensitive in analyzing phenols than phenolic acids. The limits of detection without any additional preconcentration steps (e.g. solvent evaporation) were adequate and ranged from 0.4 to 15.8 ng/mL while limits of quantitation were between 1.2 and 33.3 ng/mL. The method was applied to the determination of phenols, in spiked environmental samples and phenolic acids in aqueous infusions of commercially available pharmaceutical dry plants. The recoveries of fortified composite lake water samples and *Mentha spicata* aqueous infusions ranged from 89.3% to 117.3% for phenols and 93.3% to 115.2% for phenolic acids.

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1. Introduction

Phenolics are important substances with biological activities ranging from harmful to beneficial to human health. Phenols, which are used widely in many industrial processes such as the manufacture of plastics, dyes, drugs, antioxidants and pesticides [1–3] have been extensively found in the environment, as micropollutants. Alkylphenols and bisphenol A are well-known endocrine disrupting chemicals that have the potential to disrupt the normal functions of the endocrine system of animals [4] or can even cause cancers and heart diseases in living species including humans [5,6]. On the other hand, the role of the phenolic acids, as natural antioxidants and free radical scavengers has attracted considerable interest [7,8] due to their pharmacological behavior [9,10] and their role to inhibit the *in vitro* oxidation of human low-density lipoprotein [11].

Many analytical techniques have been employed for the determination of phenolics in aqueous samples. Despite their high polarities, the determination of such analytes is often preferred to be performed with gas chromatography, because of its inherent advantages of high resolution, rapid separation, low cost and ease of coupling with sensitive and selective detectors. Although many phenols can be analyzed directly [12], the poor chromatographic characteristics acquired deteriorate the reliability of the analysis. Thus, a derivatization step is required prior to the GC analysis. There are numerous reports on the derivatization of phenolics whereof acetylation [13–16], benzylation [17], benzoylation [18], alkylation [19–21], and silylation [22–26] are the most common. This step is often accompanied by an extraction–preconcentration step. In all cases, the acidic moiety of the substances is the target group for derivatization.

Phase-transfer catalysis (PTC) derivatization–microextraction has been considered a valuable technique since it promotes the one-step extraction–derivatization–preconcentration of phenols [27,28] between immiscible phases. PTC-assisted methylation of phenolics has already been reported by our group [29,30], exhibiting adequate sensitivity and selectivity. Phenols and phenolic acids have been easily methylated in 45 min under heating and vigorous stirring, using methyl iodide as the derivatization reagent.

Microwave irradiation energy does not induce directly chemical reactions but it is efficient for the heating of materials by the "microwave dielectric heating" effect. It is believed that the enhanced reaction kinetics are attributed to the thermal-kinetic effect caused by the rapid transfer of energy into the bulk of the reaction. Additional advantages are the "greenness" employed due to lower energy demand, the ease of utilization and the absence of heating on the vessel of the reaction.

Although microwave energy has been used extensively in organic synthesis accelerating reaction with slow kinetics under normal external heating [31], its analytical applications are scarce.

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Limited number of papers report microwave-assisted derivatization following the extraction of the analytes from the matrix [32–35] while most refer to the acceleration of analyte's extraction [36–38].

In the present work, microwave-irradiated PTC is reported, for the first time, for the rapid one-pot extraction–derivatization–preconcentration of phenolics for analytical purposes. Twenty phenols of environmental interest, including the well known endocrine disruptors, bisphenol A and pentachlorophenol along with ten selected phenolic acids possessing biological activity are easily analyzed by gas chromatography using the proposed method. Although phenols and phenolic acids are analytes hardly occurring in the same samples, the method capitalizes on the universality of this rapid one-pot derivatization–preconcentration procedure.

2. Experimental

2.1. Reagents and chemicals

The ion-pairing agents tetrabutylammonium bromide (TBAB), tetrahexylammonium bromide (THAB), cetyltrimethylammonium bromide (CTAB), as well as *n*-pentadecane (internal standard), sodium chloride, sodium hydroxide, disodium hydrogen phosphate, methyl iodide (MeI), and the phenolic compounds used for the study were all obtained from Sigma–Aldrich Hellas (Athens, Greece). The tri-phase catalysts tributylmethylammonium chloride (TBMAC), tributylmethylphosphonium chloride (TBMPC) and the ion-pairing agent hexadecylpyridinium chloride monohydrate (HPCM) were purchased from Fluka (Buchs, Switzerland). Riedel-de Haën (Seelze, Germany) supplied the GC grade solvents dichloromethane, chloroform, ethyl acetate, isooctane, toluene, *tert*-butyl methyl ether, diethyl ether and *n*-hexane. All chemicals and solvents were of the highest grade available.

2.2. Solutions

The two working standard solutions (i.e. phenols and phenolic acids) were prepared by mixing the individual standard solutions of the 30 analytes. Separate stock solutions of these analytes were acquired by dissolving (0.15–0.5 mg/mL) of each in double distilled water (DDW) whence the respective standard solutions were obtained with appropriate dilutions. The extraction solvent contained *n*-pentadecane (0.1 mM), as the internal standard (I.S.). The disodium hydrogen phosphate-NaOH buffer solution (0.1 M) was set to pH 10.5 and the ion-pairing agents were prepared in this solution, at a concentration of 0.1 M. All solutions were prepared weekly. AP-13 Extran alkaline soap (Merck, Darmstadt, Germany) was used for cleaning the glassware, which was washed subsequently with DDW and acetone and baked at 110 °C overnight.

2.3. Instrumentation-chromatographic analysis

The derivatization–extraction was done in a 10-mL Bola PTFE/TFM digestion vessel with liner (A250-04) Bohlender (Grünsfeld, Germany) using a Winson, 900W type WD900DSL23-2II household microwave oven.

The GC–MS analysis of the target compounds was performed on a Shimadzu (Kyoto, Japan) GC-17A gas chromatograph interfaced with a Shimadzu QP 5000 mass spectrometer, in the selective-ion monitoring (SIM) mode. Injections were done manually with a 10-μL microsyringe with angle-cut needle tip (0.6 mm glass barrel, i.d.; 0.11 mm needle i.d.). Separation of the analytes was done on a Supelco (Bellefonte, PA, USA) MDN-5 fused-silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) with Helium as the carrier gas (flow rate 1 mL/min). Samples were injected in the splitless mode with subsequent opening of vent valve after 1 min. The injector and detector temperatures were set at 250 and 280 °C, respectively. The GC oven temperature was programmed as follows: 60 °C for 1 min, then ramped to 100 °C at 5 °C/min, and then to 270 °C at 20 °C/min and held for 12.5 min giving a total program run of 30 min. The mass detector was operated in the electron impact (EI) mode at 70 eV and electron multiplier voltage of 1.30 kV. The mass fragments of the derivatives were obtained in the full scan mode, in the scan range from *m*/*z* 50 to 550 using a solvent delay time of 6.0 min to protect the ion multiplier from saturation. Data was collected on a personal computer using the CLASS-5000 Version 1.24 Chromatography Software (Shimadzu Chem. Lab. Analysis System and Software). Used and regenerated catalysts were photographed by scanning electron microscopy (SEM) in a Jeol JSM 5600 (Tokyo, Japan) system operating at 20 kV in order to compare the particle size and shape of each catalyst sample.

2.4. Analytical procedure

2.4.1. Microwave PTC extraction–derivatization

According to the procedural scheme, 2.5 mL of aqueous sample are placed into the 10-mL PTFE/TFM digestion vessel, followed by 0.4 mL of phosphate buffer 0.1 M (pH 10.5), 23 mg of TBMPC polymer-bound, 400 μL of extraction solvent ($CHCl_3$, containing the I.S.) and 20 μL of MeI. The vessel is tightly sealed and placed in the microwave oven. The oven is set at 40% of total microwave irradiation, for 2 min. After the 2-min reaction period, the reaction vessel is allowed to cool down for 12 min and 1 μL of the organic layer is subjected to chromatographic analysis.

Six analytical standard solutions of various concentrations were analyzed in triplicate in order to generate appropriate calibration plots for each of the analytes under study. Quantification was based on their height ratios relative to the I.S.

The heterogeneous catalyst TBMPC was regenerated according to a procedure published previously [39].

2.4.2. Sample treatment

The method was applied on surface waters and aqueous infusions. Samples originating from Lake Pamvotis and the wastewater treatment plant of the town of Ioannina, Greece, were examined for the presence of phenols. After collection (autumn 2008) the samples were filtered and stored refrigerated prior to analysis. Phenolic acids were determined in aqueous infusions prepared by commercially available dry pharmaceutical plants of *Tilia europea* and *Mentha spicata*. The infusions were prepared by adding 2 g of the dried plants to 250 mL of boiled DDW and stirred for 30 min. The plant residue was then filtered and the filtrate was analyzed for phenolic acids by the proposed method.

3. Results and discussion

3.1. Confirmation of the derivatives

The microwave-assisted ion-pair methylation of the analytes under study could be described as a S_N2 substitution reaction during which the ionized phenolic moieties form the respective ion-pair with the phase-transfer catalyst which then reacts with MeI in the organic phase. The reaction occurs instantly leading to the formation of fully methylated derivatives for all analytes under consideration, which then are liberated/extracted into the organic phase. The EI-MS data collected by the full scan analysis of a standard solution of the 30 analytes have been tabulated in Table 1. As anticipated, the hydroxyl and carboxyl groups are converted into their corresponding methyl ethers and esters. From the spectral data, it is obvious that for the derivatives formed, the base peaks coincide with the molecular ions. The $[M-15]^+$ fragment suggests

Table 1
Characteristic ions of the mass spectrum for the respective phenolic derivatives in full scan mode used for their structure elucidation and derivative identification (in parenthesis their relative abundances of the methylated phenols are given).

Phenols	M^+ or $[M+1]^+$	$[M-15]^+$	$[M-31]^+$	Other ions
o-Cresol	123 (100), **122** (30)	**107** (70)	91 (50)	77 (80)
m-Cresol	**122** (100)	**107** (30)	92 (60)	77 (80)
p-Cresol	**122** (100)	**107** (40)	91 (50)	77 (90)
Benzoic acid	**136** (30)		**105** (100)	77 (100)
3,5-Dimethylphenol	**136** (100)	121 (40)		**99** (25), 91 (100), 77 (70)
3-Chlorophenol	**142** (100)	127 (80)		**99** (80), 91 (30), 77 (40), 63 (50)
4-Chlorophenol	**142** (100)	127 (10)		112 (90), **99** (80), 77 (85), 63 (60)
2-Chlorophenol	**142** (100)	127 (70)		**99** (100), 77 (40), 63 (40)
2-Methoxyphenol	**138** (100)	**123** (50)	107 (10)	95 (80), 77 (90), 65 (50)
4-Methoxyphenol	**138** (70)	**123** (100)	107 (5)	95 (40), 63 (20)
Phenylacetic acid	**150** (20)		119 (5)	91 (100), 65 (20)
Thymol	**164** (20)	**149** (100)		135 (30), 119 (25), 105 (15), 91 (50), 77 (30)
t-Butylphenol	**164** (20)	**149** (100)		134 (5), 121 (30), 109 (15), 77 (10)
2,3,5-Trimethylphenol	**150** (20)	**135** (100)		115 (15), 91 (20)
2,4-Dichlorophenol	**176** (100)	**161** (90)		163 (65), **135** (70), 133 (100), 75 (50)
3-Nitrophenol	**153** (60)			**107** (40), 92 (80), 77 (100), 63 (60)
4-Hydroxybenzoic acid	**166** (20)		**135** (100)	107 (15), 92 (20), 77 (30), 63 (20)
t-Cinnamic acid	**162** (25)		**131** (100)	103 (85), 77 (70), 51 (80)
2-Naphthol	**158** (60)	143 (10)	128 (15)	**115** (100), 89 (10), 63 (15)
Vanillic acid	**196** (80)		**165** (100)	79 (70), 77 (50)
Homovanillic acid	**210** (40)			**151** (100), 107 (40), 91 (20), 78 (30), 65 (35)
o-Coumaric acid	**192** (20)		**161** (100)	131 (20), 118 (35), 105 (30), 89 (25), 77 (35), 63 (30)
4-Octylphenol	**220** (5)			**121** (100), 92 (5)
Pentachlorophenol	**280** (85)	**265** (100)		237 (85), 165 (50), 143 (40), 130 (60), 107 (30), 95 (70), 71 (30), 60 (60)
Syringic acid	**226** (100)	**211** (70)	195 (35)	183 (15), 168 (15), 155 (70), 137 (15), 125 (35), 107 (20), 95 (15)
Caffeic acid	**222** (100)	**207** (15)	191 (75)	165 (15), 147 (40), 133 (15), 119 (35), 103 (30), 91 (45), 77 (55)
Sinapic acid	**253** (100)	**237** (80)	221 (20)	77 (40), 63 (50)
Bisphenol A	**256** (20)	**241** (100)		133 (45), 120 (20), 77 (20)
Irgasan	**302** (100)			**252** (90), 189 (20), 159 (5), 126 (5), 75 (70), 63 (100)
Estradiol	**286** (80)			268 (20), 226 (20), 199 (40), 186 (50), 174 (50), 160 (100), **147** (100), 115 (100), 91 (100), 77 (70)

In bold characters are depicted the target ions used for the identification and quantitation in SIM analysis.

the cleavage of the O–C bond of the phenolic methyl ether and the $[M-30]^+$ corresponds to the detachment of the methoxy group of the formed ester. Either or both of these fragments can appear in the obtained fragmentograms.

3.2. Optimization of the extraction-derivatization conditions

The overall mechanism comprises several steps occurring instantly: deprotonation of the hydroxyl groups, transfer of the formed ion-pairs towards the organic phase, reaction with MeI in the organic phase or at the interphase (depending on the catalyst used) and dissolution of the derivatives into the organic phase. Obviously, the microwave-assisted PTC-based analysis of the phenolics relies upon several experimental factors needed to be optimized. The kind and concentration of catalyst, pH, organic solvent, microwave irradiation, reaction time, MeI concentration and salting out effect are the basic factors which were optimized through a two-stage sequence of variations. After a first optimization procedure, all parameters were altered again one-at-a-time, setting a second set of values. The process efficiency under different experimental conditions was assessed by the chromatographic peak height ratio (analyte-to-I.S.).

The catalysts tested were either two-phase, homogeneous (e.g. TBAB, THAB, CTAB and HPCM) or three-phase, heterogeneous (e.g. TBMAC and TBMPC). For an 8-min microwave irradiation, at 20% of total oven power, the derivatization of the thirty analytes took place in a catalyst concentration range of 10^{-4} to 10^{-1} M. It is known that strong agitation of the reaction mixture is a prerequisite for PTC processes, especially when three-phase catalysts are involved. It is also established that the heterogeneous catalysis involves swelling, mixing and diffusion during the reaction, since the catalyst movement is restricted in the interphase and the organic and aqueous reagents must be brought in contact with the catalyst cation, in sequence [27]. Evidently, the efficient mass transfer requires an agitation well-beyond a minimum stirring speed. In our case, mechanical stirring of the reactants in the microwave vessel was not applied; therefore, mixing of the reactants depended solely on the effect of microwave irradiation. During this process, the electric component of the applied electromagnetic field causes heat by dipolar polarization and ionic conduction. The irradiation causes molecular friction and dielectric loss leading to loss of energy in the form of heat, which is proportional to the ability of the polar participating reactants (i.e. phenolics and catalyst) to align with the applied field.

As expected, the extent of methylation increased with the increasing amount of PTC, while in the absence of ion-pairing agent the reaction does not take place at all. Among the homogeneous catalysts, TBAB and THAB exhibited the best reaction characteristics. The three-phase catalysts gave the highest reaction yields in the conditions tested, exceeding unexpectedly the yields acquired with the homogeneous catalysts. It seems that the energy transferred to the system due to microwave irradiation is adequate for the heterogeneous catalysts TBMPC and TBMAC to act as ion-exchange resins for anionic analytes, negating the need for external stirring to afford the desired reaction products. Throughout the study, TBMPC exhibited elevated yields of derivatization as compared to TBMAC. Thus, it was that the catalyst of choice for the next experiments. Since the catalyst is insoluble in both phases, constituting practically the interphase, it is difficult to estimate its concentration. Hence, it was chosen to present its effect in terms of its actual amount added in the aqueous sample used. Apparently (Fig. 1), 23 mg of catalyst were adequate for the procedure to be completed for the concentra-

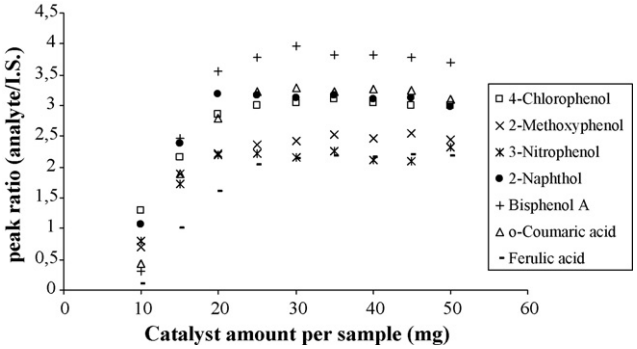

Fig. 1. Plot of TBMPC quantity vs. peak ratio for selected phenolics.

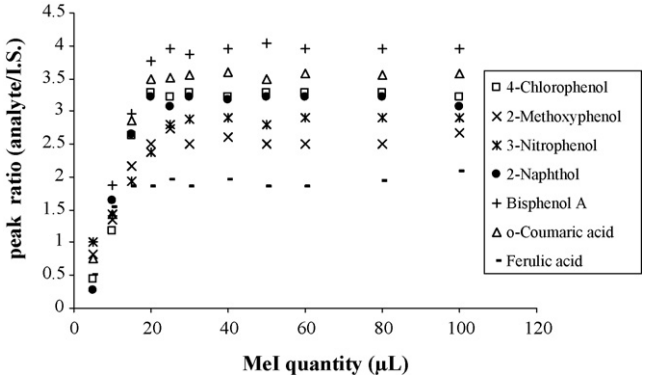

Fig. 2. Methylation yields of selected phenolics against MeI.

tions tested for phenolics. This amount of polymer-bound catalyst is 26% higher than the respective amount needed for the reaction to take place under normal heating/agitation conditions [29]. However, the main advantage of TBMPC is its ability to be recovered and regenerated, as demonstrated in a previews study. Here, this beneficial feature was further investigated by comparing SEM images acquired from primitive, microwave and thermally heated regenerated catalysts. The images (supplementary material: Fig. S1) revealed that the catalyst's particle size, shape and surface suffered no alteration after microwave heating and/or the recovery process. In concurrence with the SEM images, the microwave-reacted and regenerated catalyst showed unaffected reactivity towards extraction–derivatization–preconcentration of phenolics.

The behavior of the polymer-bound catalyst depends, to a large extent, on the nature of the organic solvent used. The organic solvent should be immiscible with water being able to promote phase separation after the reaction is terminated. It should also be polar enough in order to facilitate the dissolution of the formed ion-pair and to promote the methylation but not highly polar in order to retain the methylated derivatives within its bulk volume. Organic solvents immiscible with water such as dichloromethane, chloroform, ethyl acetate, isooctane, toluene, *tert*-butyl methyl ether, diethyl ether and *n*-hexane were used to evaluate their potency to promote the methylation reaction and uphold the derivatized analytes. The most favourable results were obtained with chloroform, which also provides greater selectivity in terms of extracting the analytes over interfering peaks, volatility and adequate immiscibility with water. As expected, the less the solvent volume used for the analysis the higher the preconcentration of the analytes achieved. Tested quantities of chloroform showed that solvent volumes smaller than 400 μL resulted in difficulties in phase separation. Thus, a solvent volume of 400 μL was considered as optimum in order to combine higher preconcentration with adequate phase separation.

Methyl iodide is a well-established and efficient methylating agent used under phase-transfer catalytic conditions for the extractive methylation of analytes with up to three hydroxyl groups. The duration of PTC methylation under conventional heating is reported from 45 to 90 min at 70–90 °C [30]. Herein, it is used for the rapid methylation of phenolics under microwave irradiation. MeI has the unique characteristic to act as derivatization reagent and as solvent since it is normally used in overwhelming excess. When analyzing the phenolic mixture in various quantities of MeI, it became evident (Fig. 2) that in contrast to conventional heating, the MeI concentration needed under microwave irradiation is significantly lower. A quantity of 20 μL was adequate to promote the methylation of the 30 analytes.

As the reactive groups are phenolics, which have to be transferred into the organic phase in the form of ion-pairs, it is easily understood that the pH of the sample should be at least two units above the p*K*a value of the weakest acid to ensure that all analytes are in their ionic form. Bearing in mind that PTCs are unstable under highly basic pH conditions [40] the pH range tested was between 4 and 11. It was found that the methylation yield of all phenolics rises at increasing pH values up to pH 10.0. Finally, a pH of 10.6 was chosen since at this value compounds with more than one reactive site can thoroughly be ionized.

Salting out effect was also evaluated and was shown that for lower salt concentrations (~3%), the overall reaction performance was not altered; at higher salt concentrations lower reaction yields were acquired. When saturated aqueous samples were analyzed, the derivatization reaction was totally inhibited as the anionic species act antagonistically to the phenolic anions towards the formation of the required ion-pairs. On the other hand, after reaction completion, the saturation of the aqueous samples with NaCl had minor effect on the extraction of the analytes into the organic solvent. Thus, the addition of salt was not judged to be necessary in the proposed methodology.

The temperature of the reaction is a rate-determining parameter influencing the reaction time and the product yield. Under normal heating conditions, increasing temperatures lead to reaction acceleration. However, polymer-bound catalysts go through degradation when high temperatures are employed. This decomposition may start from 60 °C depending on base concentration [41]. This problem intensifies when microwave irradiation is used where heating increases uncontrollably, especially in the presence of strong bases. Using 20% of the total irradiation power for various reaction times maximum reaction yields were acquired after 8 min. A four-fold acceleration of the reaction was accomplished when a 40% of the total irradiation power was used, as shown in Fig. 3. Under these conditions, the reaction reaches the maximum yield at 2 min for almost all the compounds tested. For safety rea-

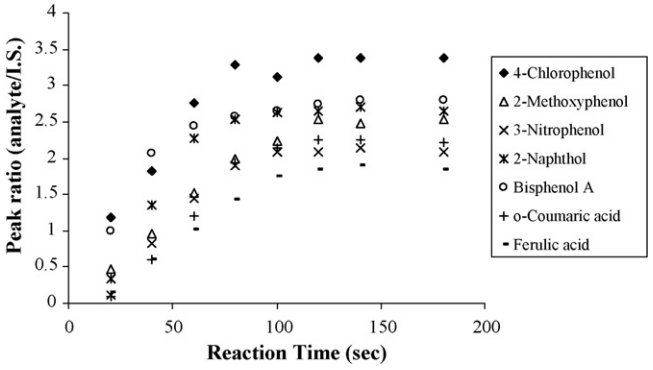

Fig. 3. Methylation yields of selected phenolics when 40% of total irradiation power of the microwave oven was used vs. reaction time.

Table 2
Analytical figures of merit of the microwave-assisted ion-pair transfer derivatization processes for the analysis of standard solutions.

Phenols	t_R (min)	R^a	DLR[b] (ng/mL)	LOD[c] (ng/mL)	LOQ[d] (ng/mL)	Within-day RSD (%)	Intra-day RSD (%)
o-Cresol	9.033	0.9953	6.2–687.0	1.3	6.2	3.3	4.8
m-Cresol	9.317	0.9901	3.7–527.3	0.9	3.7	2.9	4.1
p-Cresol	9.3650	0.9945	2.3–449.6	0.8	2.3	2.9	5.1
Benzoic acid	10.025	0.9985	15.4–634.3	3.2	15.4	3.5	6.1
3,5-Dimethylphenol	11.108	0.9933	0.8–226.9	2.4	0.8	3.3	5.8
3-Chlorophenol	11.258	0.9943	1.3–473.8	0.7	1.3	2.4	5.1
4-Chlorophenol	11.305	0.986	1.2–230.5	0.4	1.2	2.7	5.1
2-Chlorophenol	11.667	0.9934	2.5–318.3	0.7	2.5	3.1	5.5
2-Methoxyphenol	12.058	0.9941	1.5–535.4	0.5	1.5	3.3	6.2
4-Methoxyphenol	12.217	0.9888	2.2–449.3	0.8	2.2	3.1	7.2
Phenylacetic acid	12.505	0.9985	30.3–831.6	7.9	30.3	4.4	6.6
Thymol	12.767	0.9931	2.4–432.4	0.9	2.4	3.2	5.7
t-Butylphenol	12.925	0.9974	2.7–580.3	0.8	2.7	3.8	6.3
2,3,5-Trimethylphenol	13.508	0.9953	4.7–538.9	1.2	4.7	3.5	7.5
2,4-Dichlorophenol	13.742	0.9937	3.3–473.4	0.8	3.3	2.4	5.2
3-Nitrophenol	14.275	0.9953	3.5–429.9	0.8	3.5	3.3	5.6
4-Hydroxybenzoic acid	14.722	0.9975	33.3–641.5	8.1	33.3	2.8	4.6
t-Cinnamic acid	14.975	0.9987	19.2–726.3	4.2	19.2	3.1	3.9
2-Naphthol	15.358	0.993	3.3–487.4	0.7	3.3	3.6	5.1
Vanillic acid	15.663	0.9996	4.3–854.8	1.3	4.3	3.8	7.5
Homovanillic acid	16.142	0.9989	21.3–598.5	6.2	21.3	3.8	5.3
o-Coumaric acid	16.490	0.9967	16.9–618.4	3.2	16.9	3.9	4.7
4-Octylphenol	16.783	0.9923	4.2–447.9	0.8	4.2	2.4	4.4
Pentachlorophenol	17.275	0.992	2.1–482.7	0.7	2.1	2.8	5.5
Syringic acid	17.403	0.9991	51.4–593.9	15.8	51.4	3.4	6.3
Caffeic acid	18.675	0.9856	32.2–631.8	10.1	32.2	3.3	5.8
Sinapic acid	19.132	0.9989	34.6–644.3	8.6	34.6	4.1	7.8
Bisphenol A	19.647	0.998	7.5–779.3	0.9	7.5	3.8	7.5
Irgasan	20.200	0.9969	3.6–571.5	0.7	3.6	4.1	6.9
Estradiol	27.511	0.9927	10.6–665.7	3.2	10.6	4.6	7.7

[a] Correlation coefficient: calculated from three replicates at six concentration levels.
[b] DLR: dynamic linear range.
[c] Limit of detection.
[d] Limit of quantification.

sons, higher microwave irradiation power was not attempted to be used. It is noteworthy that the PTC reaction, which under external heating and rigorous agitation requires 45 min to reach its maximum yields, is considerably accelerated by at least twenty times. Thus, a 2-min reaction time utilizing the 40% of total irradiation power was considered as the optimum parameters.

Inorganic salts, humic substances and other anionic analytes, usually present in aqueous environmental samples, may interfere with the analysis deteriorating method's analytical characteristics. As was seen in the optimization study (salting out effect) inorganic anions existing in the matrix of the analysis may affect the overall procedure depending on their concentration, nucleophilicity, lipophilicity and/or affinity for the ion-pairing agent used. In order to examine the possible negative impact of interfering agents, humic acids as well as sodium phosphate, nitrate and sulfate were added to standard solutions containing the 30 phenolics. The total concentration of these anions was fixed at 20 mg/L. No particular effect on the analysis was observed leading to the conclusion that the method is tolerable against samples with normal range of interfering agents.

3.3. Method validation

3.3.1. Calibration curves

Standard composite aqueous samples were analyzed in triplicate for a range of concentrations in order to evaluate method's linearity (Fig. 4). Calibration plots were constructed by the response ratios (analyte-to-I.S.) vs. the respective amounts injected. The correlation coefficients acquired after the GC/MS (SIM) analysis of these solutions demonstrated adequate linearity ranging between 0.9856 and 0.9996 (Table 2).

Limits of detection (LOD) were estimated using $3S_b$/slope while limits of quantification (LOQ) using $10S_b$/slope of the calibration

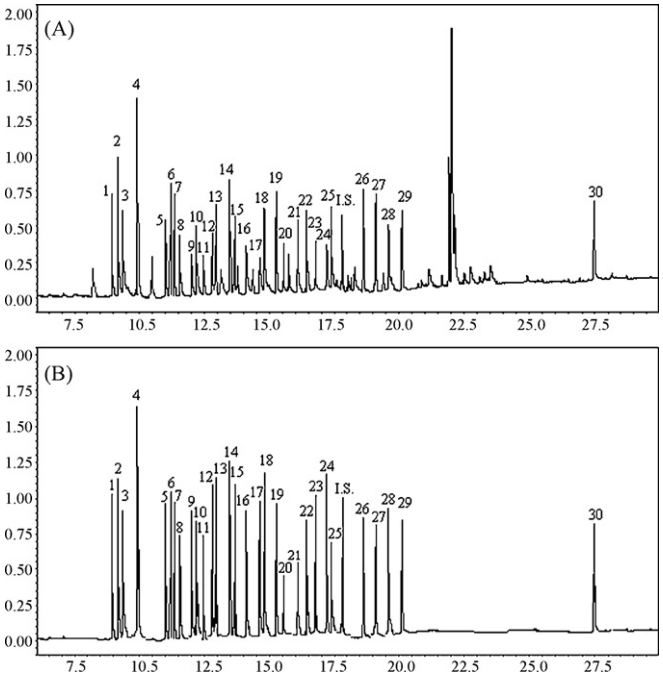

Fig. 4. Chromatographic analysis of a standard aqueous sample in (A) full scan and (B) in SIM mode (1: *o*-cresol, 2: *m*-cresol, 3: *p*-cresol, 4: benzoic acid, 5: 3,5-dimethylphenol, 6: 3-chlorophenol, 7: 4-chlorophenol, 8: 2-chlorophenol, 9: 2-methoxyphenol, 10: 4-methoxyphenol, 11: phenylacetic acid, 12: thymol, 13: *t*-butylphenol, 14: 2,3,5-trimethylphenol, 15: 2,4-dichlorophenol, 16: 3-nitrophenol, 17: 4-hydroxybenzoic acid, 18: *trans*-cinnamic acids, 19: 2-naphthol, 20: vanillic acid, 21: homovanillic acid, 22: *o*-coumaric acid, 23: 4-octylphenol, 24: pentachlorophenol, 25: syringic acid, 26: caffeic acid, 27: sinapic acid, 28: bisphenol A, 29: irgasan, and 30: estradiol).

Table 3
Analysis of wastewater treatment plant and Lake Pamvotis samples with the proposed protocol.

Phenols	Wastewater treatment plant	Lake Pamvotis				
	μg/L	μg/L	Spiked (μg/L)	Found (μg/L)	Recovery (%)	Precision (%)
o-Cresol	Detected	n.d.	22.9 527.3	21.4 566.9	93 107	3.8 3.5
m-Cresol	n.d.	n.d.	15.4 453.8	14.2 449.3	92 99	4.2 3.6
p-Cresol	n.d.	n.d.	7.9 389.2	9.2 375.9	116 97	4.8 3.9
3,5-Dimethylphenol	n.d.	n.d.	2.3 225.5	2.7 221.1	117 98	3.9 3.7
3-Chlorophenol	n.d.	n.d.	4.8 353.4	4.4 345.7	92 98	4.7 4.2
4-Chlorophenol	n.d.	n.d.	6.3 195.9	6.1 199.1	97 102	3.9 3.3
2-Chlorophenol	n.d.	n.d.	4.6 295.8	4.9 291.2	106 98	3.8 3.5
2-Methoxyphenol	n.d.	n.d.	3.3 499.2	3.1 493.3	94 99	4.7 3.9
4-Methoxyphenol	n.d.	n.d.	5.7 384.6	5.2 379.3	91 99	5.2 3.8
Thymol	n.d.	n.d.	4.3 385.2	4.6 379.4	107 98	4.7 3.8
t-Butylphenol	n.d.	n.d.	5.6 485.7	5.9 471.2	105 97	4.6 4.2
2,3,5-Trimethylphenol	Detected	n.d.	5.5 476.2	5.4 471.1	98 99	4.9 3.9
2,4-Dichlorophenol	Detected	n.d.	5.4 397.9	5.9 382.1	109 96	4.1 2.7
3-Nitrophenol	n.d.	n.d.	4.9 379.1	4.2 371.9	86 98	3.9 3.6
2-Naphthol	n.d.	n.d.	5.7 399.9	6.1 386.9	107 97	5.3 3.9
4-Octylphenol	Detected	n.d.	5.2 338.3	4.9 333.7	94 99	4.7 3.3
Pentachlorophenol	n.d.	n.d.	5.9 422.8	5.1 412.2	86 97	3.9 3.4
Bisphenol A	Detected	n.d.	13.5 665.2	12.5 644.9	92 97	5.5 4.2
Irgasan	n.d.	n.d.	6.9 492.8	6.3 483.3	91 98	5.3 3.9
Estradiol	n.d.	n.d.	15.9 578.8	14.2 558.3	89 96	6.2 3.4

Mean values of three replicates; n.d.: not detected.

curve, where S_b is the standard deviation of the blank measurements ($n = 3$). Without the use of any additional preconcentration steps, the actual LOD and LOQ values of the method were then determined experimentally and ranged from 0.4 to 3.2 ng/mL and 0.8 to 10.6 ng/mL for the selected phenols while for the phenolic acids these values ranged higher from 3.2 to 15.8 and 3.9 to 34.6 ng/mL.

The within-day repeatability ($n = 5$) and between-day reproducibility (3 consecutive days, $n = 3$) were assessed by analyzing standard solutions with phenolics with concentrations in the range of 2.0–35.0 ng/mL. The relative standard deviations obtained are given in Table 2 and were found less than 4.6% and 7.8%, respectively.

As mentioned in the introduction, PTC-assisted methylation of phenolics has already been reported by our group. A comparison between the two methods revealed that LODs and LOQs for phenols are significantly reduced [29] when microwave irradiation is used, but for phenolic acids the analytical features are similar (e.g. 4-hydroxybenzoic acid, *trans*-cinnamic acid) or worse (e.g. homovanillic acid and syringic acid) [30]. Although microwave irradiation accelerates the reaction, when one hydroxyl group is present, it seems that a second S_N2 reaction is not that favoured.

3.3.2. Sample analysis

The practicability and applicability of the method were confirmed by analyzing different matrices, where phenolic compounds play contradictory roles: water samples from Lake Pamvotis and wastewater treatment plant of the city of Ioannina and aqueous infusions from dried plants of *M. spicata* and *Sideritis cretica*. The environmental samples were collected, filtered and analyzed without any other kind of treatment. Amounts close to the LODs of *o*-cresol, 2,4-dichlorophenol, 2,3,5-trimethylphenol, 4-octylphenol and bisphenol A were found in wastewater samples while in the Lake samples the phenols under study were absent (Table 3).

Table 4
Analysis of *Sideritis cretica* and *Mentha spicata* aqueous infusions with the proposed protocol.

Phenolic acids	*Sideritis cretica*	*Mentha spicata*				
	mg/L	mg/L	Spiked (mg/L)	Found (mg/L)	Recovery (%)	Precision (%)
Benzoic acid	n.d.	n.d.	0.033	0.038	115	4.2
			0.532	0.541	102	3.9
Phenylacetic acid	n.d.	n.d.	0.056	0.053	95	4.8
			0.767	0.774	101	3.3
4-Hydroxybenzoic acid	3.421 ± 0.006	0.865 ± 0.004	0.046	0.918	101	4.6
			0.552	1.35	95	3.6
t-Cinnamic acid	0.886 ± 0.003	0.45 ± 0.008	0.034	0.465	96	5.3
			0.353	0.841	105	3.9
Vanillic acid	0.096 ± 0.005	n.d.	0.015	0.023	153	5.5
			0.784	0.754	96	3.9
Homovanillic acid	0.765 ± 0.008	0.41 ± 0.006	0.051	0.432	94	5.2
			0.464	0.845	97	4.2
o-Coumaric acid	0.051 ± 0.007	0.122 ± 0.007	0.034	0.149	95	4.8
			0.453	0.561	98	3.1
Syringic acid	1.369 ± 0.006	n.d.	0.075	0.071	95	3.9
			0.575	0.538	94	3.3
Caffeic acid	6.631 ± 0.008	5.6 ± 0.009	0.049	5.50	97	4.2
			0.477	5.94	98	3.8
Sinapic acid	n.d.	n.d.	0.058	0.063	109	3.8
			0.569	0.531	93	3.2

Mean values of three replicates; n.d.: not detected.

Chromatographic analysis of the infusions revealed the presence of several phenolic acids as depicted in Table 4. Samples from Lake Pamvotis and *M. spicata* were chosen as matrices for spiking with two different analyte concentrations (low-concentration spiking chromatograms are depicted in supplementary material: Fig. S2). The samples were analyzed in triplicate in order to assess the recovery of the method as well as the respective relative standard deviations. Standard solutions were run in between experiments in order to check system's behavior and chromatographic performance. The almost quantitative analyte recoveries and the absence of interference from the complex matrices point out the satisfactory analytical features of the method. The repeatability and accuracy together with the ease of manipulation and rapid derivatization–extraction characteristics demonstrate the high potentials of the method.

4. Conclusions

The transformation of phenolics to their respective methyl ethers and/or esters is a popular and convenient procedure for their gas chromatographic determination. Phase-transfer catalysis facilitates the direct one-pot extraction–derivatization–preconcentration of acidic moieties under intensive heating/agitation conditions. Changing from the normal heating and agitation of the reaction to the microwave irradiation leads to a significant acceleration of the reaction, especially when a three-phase catalyst is used. The polymer-bound tributylmethylphosphonium chloride under microwave irradiation proved to be superior as a catalyst and reusable. Using a low-cost microwave oven, 30 phenolics were rapidly and reproducibly derivatized and analyzed with gas chromatography. Compared to normal heating, the proposed method offers reduced reaction times, and increased sensitivity for phenols while similar or worse sensitivity was recorded for phenolic acids. Finally, the practicability and applicability of the method were confirmed by analyzing various samples of environmental and pharmacological interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2009.12.016.

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