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MultiSimplex optimisation of purge-and-trap extraction of phenols in soil samples

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Abstract

Purge-and-trap extraction and GC–FID determination of phenols from soil samples has been optimised making use of the simplex method implemented in the MultiSimplex program for four phenols (2-chlorophenol, 2-methylphenol, 2-nitrophenol and 2,4-dichlorophenol). The experimental variables studied were the sample heating time and temperature, the purge time and the concentration of sodium chloride. Once the optimum conditions were established the reproducibility of the extraction yield was tested by means of repeated analysis on different samples. These results were compared with previously optimised microwave-assisted extraction and Soxhlet extraction.

Keywords: Purge-and-trap methods; Simplex optimisation; Phenols

1. Introduction

Phenol and its derivatives are widely used in the chemical industry for the manufacture of polymers, textiles, drugs, resins, dyes, detergents, explosives, stabilisers and antioxidants [1]. Furthermore, phenolic compounds have substantial applications in agriculture as herbicides, insecticides and fungicides, thus becoming potential pollutants of soils and of surface and underground waters owing to their highly hydrophilic nature [2]. Moreover, phenols have a highly toxic character and it is well known that these substances exhibit properties that are hazardous to human health [3]. Due to their toxicity and presence in the environment, the US Environmental Protection Agency (EPA) includes 11 phenols among the main environmental pollutants [4].

Different methods for the extraction of organic pollutants in soil samples are shown in the literature: microwave-assisted extraction (MAE) [5,6], accelerated solvent extraction (ASE) [7,8] or supercritical fluid extraction (SFE) [9], for instance. But not many works have studied the headspace-gas chromatography (HS-GC) for the determination of these substances [10] and no work on dynamic headspace or purge-and-trap for the determination of phenols in soil samples has been found in the literature.

In order to perform the experimental studies for the optimisation of the purge-and-trap determination of phenols in soil samples the simplex method implemented in the Multisimplex program [11] was selected among other experimental approaches like experimental design [12]. MultiSimplex is designed as a true multivariate non-linear optimisation tool that combines the modified simplex method [13]

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with the fuzzy set theory [14] by means of the membership functions providing an efficient and flexible tool for handling different and conflicting optimisation criteria (i.e., maximisation, minimisation and target values). Different response variables, with separate scales and optimisation objectives can then be combined into a joint response measure called the aggregated value of membership. In fuzzy set theory the term ''target'' can be represented with a characteristic function varying with the response variable. This function, varying between 0 and 1, is the membership function of the variable in question. The higher the membership value is, the closer to the optimum the simplex is. Therefore, the use of this algorithm allows the evaluation of the multivariate response surface without any previous knowledge about it.

In this study the overall maximisation of the extraction yield for all the phenols was aimed and sample heating time and temperature, purge time and the concentration of sodium chloride were considered as the most significant variables. Meanwhile, since trap temperature, desorption time or desorption temperature are seldom changed they were fixed along all the experiments. For aqueous samples the salting-out effect always enhances the partitioning of the compounds into the vapour phase from solution but this is not always the case for soil samples where salting-out effect can enhance matrix–analyte interactions as well and thus provide lower efficiencies depending on the carbon content of the soil [15]. Due to this reason, the concentration of the sodium chloride solution was studied as another variable in the optimisation procedure because it could not be foreseen beforehand if the salting-out effect would have a positive or negative effect in the extraction efficiency for the selected soil.

2. Experimental

2.1. Purge-and-trap extraction

Purge-and-trap extractions were performed with a Hewlett-Packard purge-and-trap Concentrator (Avondale, PA, USA) coupled to a Hewlett-Packard 6890 Series GC instrument equipped with a flame ionisation detector. A 0.5-g aliquot of laboratory prepared soil with high phenolic content was accurately weighed in the test tube for soils. 10 ml of a sodium chloride solution (the value of the concentration at each solution was suggested by MultiSimplex), 50 μl of 2-fluorophenol (internal standard) and 10 μl of sulphuric acid were added. The sample vial was immediately capped and stirred gently. The sample was firstly prepurged using a needle sparger with helium (N-50) to get an inert atmosphere and then heated at a temperature and during a heating time suggested by MultiSimplex. Afterwards, the sample was purged with helium at a flow-rate of 40 ml min^{-1} for a time also suggested by MultiSimplex and the compounds were trapped in a Vocarb 3000 (Supelco, Bellefonte, PA, USA) trap which was kept at ambient temperature. Finally, the trap was desorbed at 250°C for 6 min and then baked at 260°C for 4 min.

2.2. Reagents and chemicals

Phenol standards were supplied as follows: phenol and 4-methoxyphenol by Merck (Darmstadt, Germany), 2-chlorophenol and 2,4-dichlorophenol by Aldrich (Dorset, UK) and 2-nitrophenol and 2-methylphenol by Fluka (Buchs, Switzerland). All compounds were reagent grade and the purities were stated to be higher than 99%. An aqueous standard solution (800 μg ml^{-1}) in Milli-Q water for phenol, 2-chlorophenol, 2-methylphenol, 2-nitrophenol and 2,4-dichlorophenol was prepared by weighing an appropriate amount of the standards. Another standard solution (800 μg ml^{-1}) which was only used for the sample fortification was prepared in hexane. The EPA 8040 surrogate standard mix which contained 2-fluorophenol (internal standard) and 2,4,6-tribromophenol at 2000 μg ml^{-1} was supplied by Supelco. Sodium chloride (purissimum) and sulphuric acid were purchased from Panreac (Barcelona, Spain). All solutions were stored at 5°C in the dark. All volumetric glassware was grade A and was calibrated at laboratory temperature.

Optimisation experiments were performed using a clay nature soil from an industrial downfall (Metalquímica del Nervión, Bilbao, Spain). The sample was ground, sieved and dried following the ISO 11464 Norm [16] for homogeneity. A 50-g portion of soil, which was checked to be free of phenols, was

analytically weighed and 5 ml of 800 μg ml^{-1} stock solution was added. Afterwards, acetone was added to completely cover the soil and the mixture was left stirring for at least 24 h and was stored in the fridge for 48 h before it was dried in an oven at 40°C. Finally, the soil was homogenised with a mortar, bottled and stored in the fridge. In order to avoid confusion with spiked samples, the samples prepared in this way were designated as laboratory prepared samples. The concentrations in the soil, on the basis of added amounts, were 79.4, 82.6, 96.4, 83.6 and 83.2 μg g^{-1} for phenol, 2-chlorophenol, 2-methylphenol, 2-nitrophenol and 2,4-dichlorophenol, respectively.

2.3. Calibration of the purge-and-trap

Once the extraction step was optimised the calibration of the analytes was performed. 0.5 g of phenol free soil were weighed and 10 ml of different stock standard solutions which contained, apart from the target compounds, the same amount of 2-fluorophenol (internal standard) and sulphuric acid as the extracts were analysed under the optimum conditions mentioned above. In relation to the chromatographic conditions, they were as follows: a 30 m×0.32 mm I.D., 0.25 μm film thickness HP-5 fused-silica capillary open-tubular column which was held at 80°C for 2 min, increased at 20°C min^{-1} to 100°C, where it was held for 1 min then, the temperature was increased at 10°C min^{-1} to 250°C where it was held for 5 min was employed. The carrier gas was helium (N-50) at a flow-rate of 3.45 ml min^{-1} and a pressure at the column head of 10 p.s.i. (1 p.s.i.= 6894.76 Pa). The injector was kept at 250°C and set in the split mode. The detector was kept at 280°C.

Table 1 summarises calibration ranges and statistical parameters related to the calibration. Both, the limit of detection and the limit of determination were estimated from the average response of four blank samples. Fig. 1 shows a chromatogram of one of the trials.

3. Results and discussion

3.1. MultiSimplex optimisation

The performance of MultiSimplex is fairly easy to follow. The first step consists on the definition of the optimisation project which includes the control variables, their reference values and their variation step; the response variables, their optimisation objectives, their influence on the joint response, and finally how the membership functions are transformed. After the initial trials, the simplex method is sequential, with addition of one new trial at a time and the evaluation of the control variables. The optimisation procedure includes a revaluation rule which means that every certain number of experiments (often a complete simplex) a previous trial is repeated experimentally. Therefore, the effect of other sources of variation might be considered in this way. Since the simplex procedure can extend for a rather long time and in order to ensure the repeatability of the instrumental responses a reference liquid sample was extracted and analysed under fixed conditions. The optimisation process ends when the optimisation objective is reached or when the responses cannot be improved further [11].

The variables considered in the optimisation were heating temperature, heating time, purge time and the concentration of sodium chloride. The reference values given to MultiSimplex were a heating tem-

Table 1
Calibration ranges and statistical parameters of the calibration curves

	2-Chlorophenol	2-Methylphenol	2-Nitrophenol	2,4-Dichlorophenol
Calibration range (μg)	4.6–45.2	4.6–40	4.2–42.6	4.2–42.2
No. of data points	6	6	6	6
D_{rf} [a]	2.68±0.07	0.48±0.02	0.54±0.09	0.92±0.04
r^2	0.979	0.959	0.900	0.936
Detection limit (L_b+3S_b) (μg)	0.2	0.6	0.9	1.2
Quantification limit (L_b+10S_b) (μg)	0.6	1.2	2.0	2.9

[a] $D_{rf}=\frac{Rf_i}{Rf_{I.S.}}$

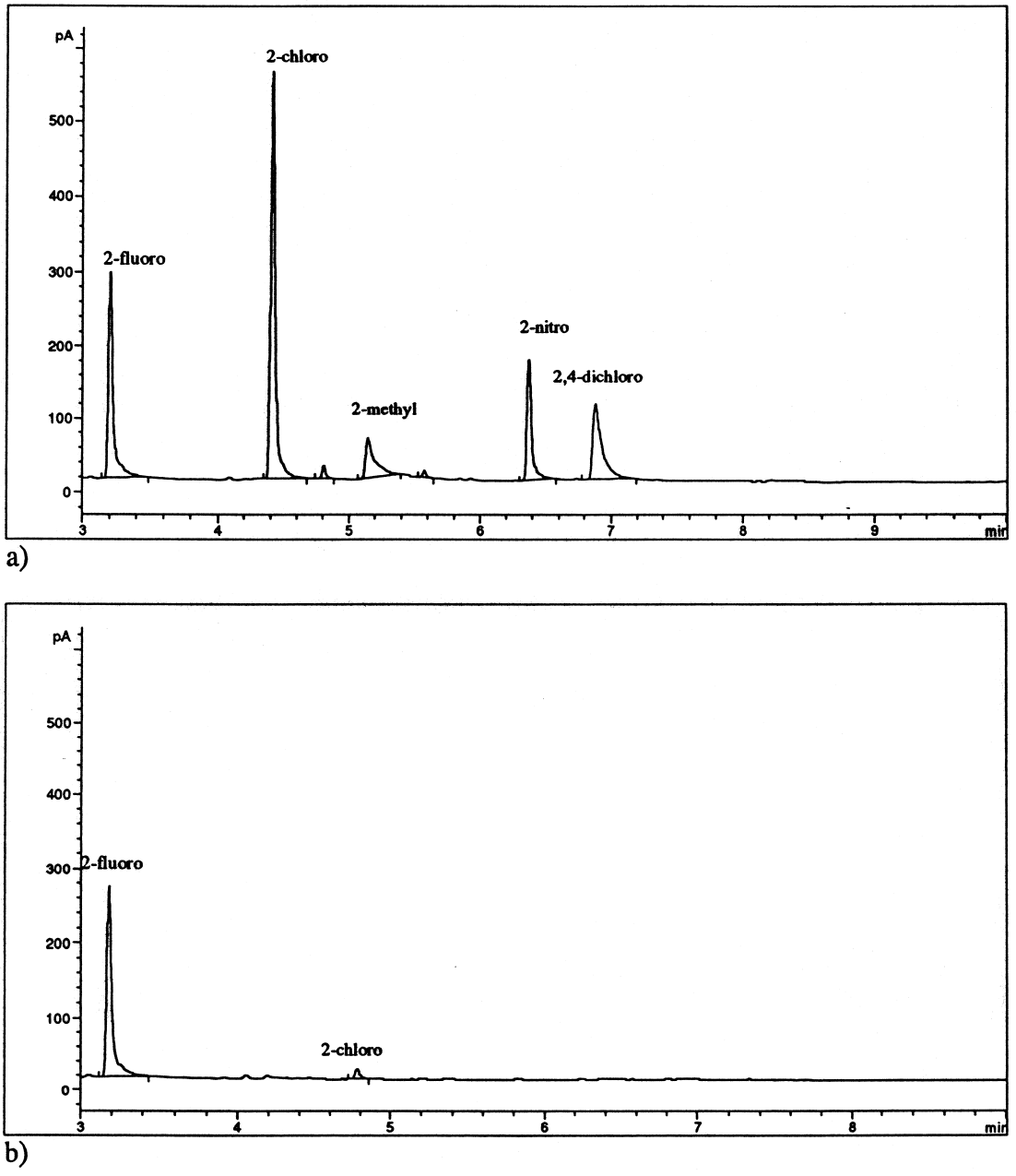

Fig. 1. GC–FID chromatograms of (a) a laboratory prepared sample and (b) blank sample.

perature of 75°C for 17.5 min, a purge time of 10 min and 2.5 mol l^{-1} sodium chloride. The step sizes given to MultiSimplex were 7.5°C, 3.5 min, 1.5 min and 0.7 mol l^{-1} for heating temperature, heating time, purge time and sodium chloride, respectively. The experiments suggested by MultiSimplex and results obtained are given in Table 2. As can be seen, the factor space covers almost the complete feasible region defined by the instrumental and chemical constraints.

Table 2
Matrix of the experiments suggested by MultiSimplex, the measured areas for each phenol and the membership value given by MultiSimplex

No.	Heating temperature (°C)	Heating time (min)	Purge time (min)	NaCl (mol l^{-1})	2-Chloro-phenol	2-Methyl-phenol	2-Nitro-phenol	2,4-Dichloro-phenol	Current membership
1	83	14.0	8.5	1.1	702.6	108.5	365.0	116.2	0.003
2	83	21.0	11.5	1.1	946.9	123.4	441.3	155.6	0.010
3	83	14.0	11.5	1.1	1351.2	180.6	358.6	139.8	0.022
4	68	21.0	8.5	3.9	1831.2	290.9	882.5	272.9	0.351
5	68	14.0	11.5	1.1	678.2	60.3	619.7	159.6	0.030
6	68	21.0	13.0	3.9	1100.9	118.3	351.3	179.3	0.010
7	60	14.0	10.8	5.0	1981.7	128.9	742.8	265.1	0.178
8	71	10.5	8.2	2.5	937.0	93.7	655.9	207.6	0.048
9	51	15.8	8.0	2.5	687.3	138.3	460.7	84.5	0.009
10	82	14.0	11.5	3.9	1773.3	240.6	207.2	296.3	0.133
11	73	15.8	8.0	5.0	1979.3	439.0	616.2	364.6	0.507
12	70	21.9	11.2	5.0	1914.1	336.4	289.3	346.0	0.386
13	53	22.4	7.8	5.0	993.8	116.6	197.4	188.6	0.008
14	82	14.0	11.5	3.9	1654.6	252.3	419.4	519.0	0.330
15	86	22.4	8.8	3.9	1660.8	178.2	362.7	267.8	0.092
16	60	14.0	10.8	5.0	1970.7	184.1	718.6	259.7	0.386
17	53	22.4	7.8	5.0	998.8	192.7	392.2	200.2	0.024

The response values for phenol are not included in Table 2 since no recovery of phenol was observed at all. This fact happened not only with soil samples but also when dealing with aqueous standard solutions no response was obtained at all. Different traps were tried just in case phenol was irreversibly retained but no improvement was achieved.

After 14 runs MultiSimplex started to suggest already given experiments while the membership function showed almost constant values which means that the simplex was around the objective. Therefore, it was decided to stop the experimentation at run number 17 since the conditions for experiment number 18 were those of experiment number 11.

According to the results obtained it could be concluded that the optimum conditions for the purge-and-trap determination of 2-chlorophenol, 2-methylphenol, 2-nitrophenol and 2,4-dichlorophenol were a 8 min purging after a sample heating at 73°C for 15.8 min and a sodium chloride solution of 5.0 mol l^{-1}.

In order to aid the description of the response surfaces, the experimental values given in Table 2 were fitted to a polynomial function [6,17] with the non-linear analysis program NLREG [18]. As it has been described before [6,17], all the parameters with a probability of being null higher than 10% were systematically eliminated from the general model, therefore this function can be slightly different for each phenol but in all cases they explain up to 99.7% of the total variance. Therefore, the use of the fitting function is equivalent to the experimental values. The values of the parameters obtained from a previously normalised values are given in Table 3. Since there were four variables and all of them showed an effect on the extraction efficiency, the graphical analysis of the response surface is hardly done. This fact is one of the reasons to follow MultiSimplex approach instead a graphical inspection in order to obtain the optimum conditions since it takes into account all the variables at the same time as well as the responses of all the compounds.

The performance of the MultiSimplex optimisation is very satisfactory. Compared with an experimental design the amount of experiments required to define the response surface is lower. In case of a complete composite design the amount of experiments would have been at least 25 and if a single degree of fractionality were introduced the experiments would have decreased to 17. In any case, none of the experimental designs would have given the optimum conditions. In this particular study, by means of

Table 3
Values of the parameters obtained from regression analysis of normalised experimental values

Compound	β_1	β_2	β_3	β_4	β_{12}	β_{13}	β_{14}	β_{23}	β_{24}	β_{34}	β_{11}	β_{22}	β_{33}	β_{44}
2-Chloro-phenol	−2.9±0.2	0.27±0.03	–	2.1±0.4	0.046±0.003	−0.042±0.006	0.09±0.02	0.024±0.001	−0.045±0.004	0.11±0.01	–	$(-6.6\pm0.4)10^{-3}$	−0.051±0.004	−0.14±0.03
2-Methyl-phenol	−4.1±0.5	0.35±0.08	0.8±0.2	–	0.053±0.006	–	–	0.015±0.002	−0.015±0.005	0.10±.02	–	$(7.2\pm0.1)10^{-3}$	−0.06±0.01	–
2-Nitro-phenol	−7.2±1.4	1.1±0.2	–	–	0.04±0.01	−0.17±0.03	0.28±0.06	0.030±0.004	−0.042±0.009	–	0.28±0.07	−0.014±0.002	−0.015±0.005	–
2,4-Dichloro-phenol	−0.6±0.1	–	–	–	0.010±.002	−0.006±0.003	–	–	0.009±0.001	–	–	$(-5\pm1)10^{-4}$	–	–

MultiSimplex 17 experiments were performed and both, the optimum and the response surface were obtained.

3.2. Evaluation of repeatability of purge-and-trap determinations

In order to evaluate the repeatability of the measurements for purge-and-trap, five aliquots of the sample were extracted each day and this procedure was repeated during three days under the optimum conditions mentioned above (8 min purge after 15.8 min heating at 73°C, concentration of sodium chloride 5 mol l^{-1}). The results obtained during the three days together with the F values are given in Table 3.

The results were analysed by means of analysis of variance (ANOVA) of the set of experimental data in Table 4. It could be observed, for a degree of confidence of 95%, that there were no significant differences for samples extracted among days. In case of samples extracted within-day 2-chlorophenol and 2,4-dichlorophenol showed slightly significant differences for the degree of confidence established.

As a consequence, and as mentioned in previous works [6,19], it was decided to express the total variance of the measurements as the sum of variance due to the analysis, the variance within-day and, in spite of not being significant the variance among days as indicated in Eq. (1)

$$s_{tot}^2 = s_a^2 + s_{w.d}^2 + s_{a.d}^2 \quad (1)$$

Table 5
Values for the relative standard deviations

Compound	RSD (%)			
	Analysis	Within-day	Among days	Total
2-Chlorophenol	0.2	18.7	11.0	21.8
2-Methylphenol	0.4	16.3	11.8	20.2
2-Nitrophenol	0.3	17.7	10.3	20.5
2,4-Dichlorophenol	0.3	17.4	5.7	18.3

where s_{tot}^2 is the total variance, s_a^2 is the variance due to the analysis, $s_{w.d}^2$ is the variance within days and $s_{a.d}^2$ is the variance among days.

Table 5 gives the relative standard deviations (RSDs) for the analysis, for samples within-day, samples among days and total RSDs estimated from Eq. (1). The total RSD is a bit high for all the compounds (~20%) but it should be taken into account that the percentage of recovery is rather low, 58%, 46%, 45% and 30% for 2-chlorophenol, 2-methylphenol, 2-nitrophenol and 2,4-dichlorophenol, respectively.

3.3. Laboratory prepared samples versus spiked samples

When no reference materials exist for performance evaluation, soil samples containing the analytes of interest must be prepared in the laboratory. Typically this is performed by adding the analyte in a carrier solvent to the sample matrix and after this sample

Table 4
Percentage of recoveries for 15 samples of each phenol determined during three days by purge-and-trap

Sample No.	Day No.											
	2-Chlorophenol			2-Methylphenol			2-Nitrophenol			2,4-Dichlorophenol		
	1	2	3	1	2	3	1	2	3	1	2	3
1	48	48	36	43	53	42	43	53	30	22	27	22
2	59	69	54	40	47	38	40	47	39	24	28	27
3	65	54	64	36	55	63	36	55	57	28	35	38
4	74	50	60	34	52	53	34	52	55	29	36	34
5	78	65	45	45	35	50	45	35	48	36	30	27
$F_{\text{within-day}}$ [a]		5.41			0.65			0.43			4.1	
$F_{\text{among days}}$ [b]		0.16			2.12			1.21			1.1	

[a] Within-day $F_{crit,4,8}^{0.05} = 3.84$.
[b] Among days $F_{crit,2,8}^{0.05} = 4.46$.

spiking the analysis are typically performed in a continuous sequence. This spiking and recovery procedure has only a very short analyte–soil contact period (i.e., minutes), so it limits analyte distribution and sorption. Thus, this procedure tests mainly the instrumental determination step and largely ignores analyte extraction from soils or sediments [20]. For this reason, it was considered more realistic to prepare a soil sample with phenols as it was mentioned above (see Section 2.2) instead of the spiking procedure.

Blank soil samples were spiked with the standard stock solutions in a way that the final concentrations were similar to those in the laboratory prepared soil. Table 6 shows the average recoveries obtained for a laboratory prepared soil sample (15 replicates) and spiked samples (five samples). It could be observed that the recoveries obtained for spiked samples are higher than those obtained for laboratory prepared samples. It could be concluded that as mentioned by Hewitt [20] spiking methods do not consider interactions between the analyte and the matrix and are not therefore suitable for the simulation of a real sample.

Table 6
Average recoveries for laboratory fortified samples and spiked samples

	Prepared samples	Spiked samples
2-Chlorophenol	58±13	89±5
2-Methylphenol	46±9	124±15
2-Nitrophenol	45±9	113±8
2,4-Dichlorophenol	30±5	87±10

3.4. Purge-and-trap versus MAE and Soxhlet extraction

Three samples from the laboratory prepared soil were extracted under the optimum conditions obtained for MAE and Soxhlet extraction [6]. Fig. 2 compares the recoveries obtained with each of the three determination procedures. For many of the phenols the recoveries obtained with MAE or Soxhlet extraction are higher than those obtained for purge-and-trap determination, except for 2-chlorophenol whose recoveries by MAE and purge-and-trap are comparable. It should be taken into account that 2-chlorophenol is the most volatile of the phenols studied and is liquid at room temperature. Although the recoveries obtained by purge-and-trap are lower than the recoveries obtained by MAE or Soxhlet, it should be noted that these recoveries obtained by purge-and-trap could be enough since the determination limits of purge-and-trap are in the range of 0.6–2.9 $\mu g\ g^{-1}$.

Apart from extraction efficiencies, there is another fact that is important when comparing extraction methods: sample handling and time and reagents spent in sample preparation. When determining phenols by purge-and-trap, handling requirements are lower, just weighing the sample. When extracting phenols by MAE or Soxhlet extraction other steps like filtration of the sample and concentration are necessary. These last steps, as well as time consuming, add errors to the analytical procedure due to

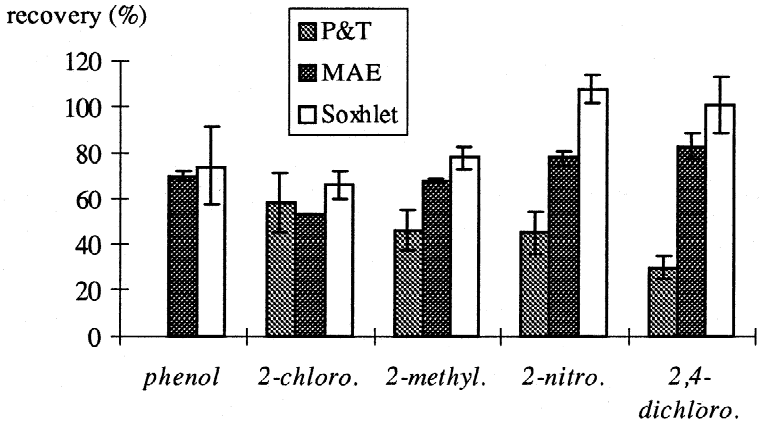

Fig. 2. Comparison of the recoveries obtained with the three different determination procedures.

Table 7
Reagent and time requirements for purge-and-trap, MAE and Soxhlet determination of phenols

Purge-and-trap	MAE	Soxhlet
10 ml Milli-Q water	15 ml acetone–hexane (1:1)	200 ml acetone–hexane (70:30)
Prepurge 3 min		
Heating time 15.8 min	MAE extraction 16.5 min	Soxhlet extraction 8 h
Purge 8 min		
Desorb 6 min	Cooling and filtering 10 min	Concentration at rotary evaporator 15 min
Bake 4 min	Concentration with nitrogen blowdown 10 min	Concentration with nitrogen blowdown 10 min
Chromatographic determination is done simultaneously with the desorption and baking steps	Chromatographic determination 10 min	Chromatographic determination 10 min
Total time 36.8 min	Total time 46.5 min	Total time 8.5 h

sample losses. Table 7 summarises the reagents and the time necessary in the different steps of the three methods proposed for the phenol determination.

Strictly speaking, the extraction step lasts more or less the same in both MAE and purge-and-trap determination methods but, whereas in MAE the presence of the operator is necessary during the whole procedure, in purge-and-trap determinations the operator only has to load the sample in the purge-and-trap concentrator. Soxhlet extractions are, in this sense, very time consuming.

Concerning the reagents needed in the three procedures, Soxhlet extraction requires larger volumes of solvents while MAE and purge-and-trap determinations require similar solvent volumes. The most significant difference is the nature of the solvents because MAE and Soxhlet extraction generally make use of acetone–hexane mixtures while purge-and-trap only requires deionised water.

As a result of this study it could be concluded that purge-and-trap determination is an alternative determination method for many phenolic compounds and comparable to MAE extraction in time and reagent consumption.

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