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A Continuous Preconcentration/Extraction Method for Organic Trace Analysis by Capillary Gas Chromatography

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Key Words

Continuous extraction
Trace analysis
Capillary Gas chromatography
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Summary

A slightly modified steam distillation-extraction device is described for the continuous extraction and preconcentration of organic traces in aqueous samples, prior to capillary G.C.-analysis.

The quantitative performance, both theoretically and practically, is studied using phenols as the test substances. The final recovery is determined by the flow-ratio of the water and the extracting solvent and by the extraction coefficient.

The process is found to be highly reproducible even at low concentration levels (ppb's). Using 30 ml. samples with a concentration of 30 ppb (1:10^9), 100 % recoveries are obtained for the phenolic substances studied, with a relative standard deviation of about 3 %, both for methylene chloride and ethylacetate as the extracting solvents. Using methylene chloride as the extracting solvent, for phenol a maximum recovery of 80 % was obtained.

Introduction

Steam distillation-extraction was introduced by Likens and Nickerson [1] in 1964. In a continuous process the condensing water vapour is extracted by the condensing solvent vapour. Godefroot et al. [2] developed a micro version for analytical applications. Recently Rijks et al. [3] discussed the possibilities and limitations of steam distillation-extraction as a preconcentration technique for trace analysis of organics by capillary gas chromatography. The effect of different process factors on the recovery was studied. It was shown that high extraction efficiencies are obtained for most types of organic compounds in a relatively short time.

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The limiting factor, especially for polar and high boiling compounds, was found to be the gas-liquid distribution constant or the distillation step. Polar compounds hardly reach the extraction part of the apparatus. Long process-times are required to achieve reasonable recoveries, although the extraction coefficient would predict a higher yield.

This problem can expectedly be avoided by a direct introduction of the aqueous sample onto the top of the condensor (cold finger). Simultaneously a continuous, direct extraction of the aqueous sample with the condensing extracting solvent is achieved. The limiting distillation step is avoided so that the enrichment of polar and high boiling compounds is facilitated. Only a slight modification of the steam distillation-extraction device is required to make it suitable for both steam distillation-extraction and continuous liquid extraction.

Experimental

Chemicals

The solvents, methylene chloride and ethylacetate, used for the extraction were redistilled in an all glass equipment to obtain a sufficiently pure grade.

The test mixture consisted of phenol and the mono-, and dimethyl substituted phenols with boiling points between 180 and 225 °C. The concentration of the stock solution was about 0.2 % (w/v) per component in methylene chloride. The normal alkane n-C11, in a 1ppm(v/v) concentration in both the solvents, served as an internal standard.

The synthetic samples and the reference solutions were prepared by adding an appropriate aliquot of the stock solution either to doubly distilled water or to the solvent which already contains the internal standard.

Gas chromatography

All analyses were performed on a Carlo-Erba 4160 gas chromatograph (Carlo-Erba Strumentazione, Milan, Italy), used in the splitless-mode. The injector and detector temperatures were both 250 °C. Helium was used as the carrier gas. A home-made glass capillary column (L = 30 m; i.d. = 0.25 mm; stationary phase = OV1) was used throughout the measurements.

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Splitless sample introduction was performed at an oven temperature 20 °C below the boiling point of the solvent; after the splitless-time of 15 seconds the oven temperature was programmed to 115 °C at a programming rate of 5 °C/min.

Determination of the extraction coefficient

In order to enable the comparison of the theoretical and experimental recoveries the extraction coefficients of the phenolic compounds were determined as follows. Equal volumes (10 ml.) of doubly distilled water and extracting solvent were given to a 50 ml. separation funnel, together with an appropriate aliquot of the phenolic sample to reach a 1 ppm concentration after complete extraction. The extracting solvent already contained n-C11 (1 ppm) as an internal standard. The solubility of this internal standard in water is extremely low so the distribution constant is assumed to be infinite.

The organic layer was analysed after intensive mixing and equilibration. The extraction coefficients were calculated as the ratio of the concentration in the extract relative to the difference in the original concentration between an appropriate reference solution and the extract.

Equipment for continuous liquid extraction

The apparatus used for this study, which is a slight modification of the device presented by Rijks et al. [3], is shown in Fig. 1. The condensor is provided with a glass helix in order to enlarge the contact time between the condensed solvent and the sample. The aqueous sample is introduced onto the top of the condensor. The sample container (A') is equipped with a teflon lined stopcock to allow an adjustable small sample feed.

The device presented here was specially constructed for this study. Nevertheless, the steam distillation-extraction device (Fig. 1.2) can also be used by simple exchange of the sample flask by a glass beaker for collection of the extracted sample and shortening of vent H.

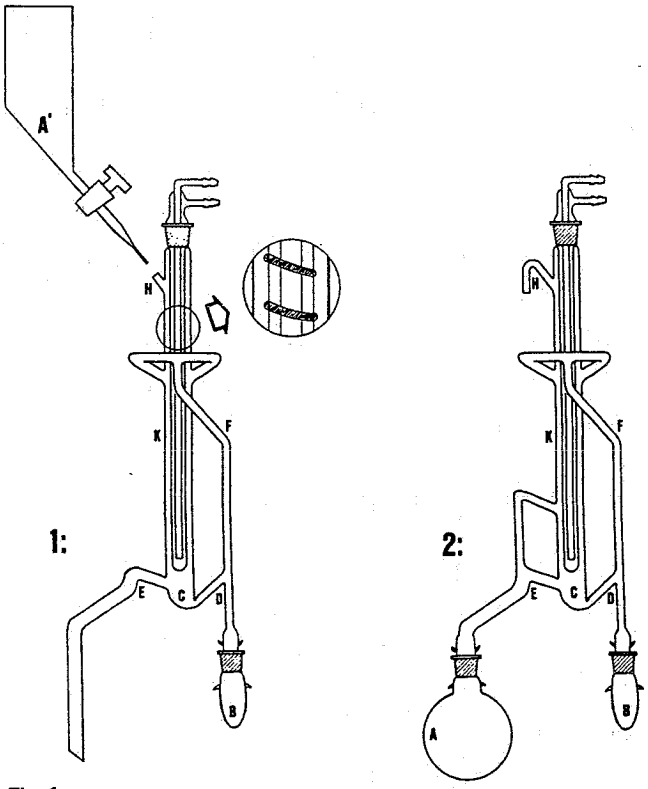

Fig. 1

1: Design for a continuous liquid extraction device. The glass helix around the condensor is enlarged in an exposed view.

2: Design of the original steam distillation extraction device.

Procedure

Of both the solvent and the doubly distilled water 1.5 ml were introduced through the side arm H (c.f. Fig. 1) in the separation chamber C after which both side arms, D and E, automatically were filled. The solvent flask B was filled with 1 ml. solvent containing the internal standard. One cleaned boiling chip was added. The solvent container was heated by means of a home-made small basket of heating wire. Different solvent flows could be established by varying the electrical voltage. When using ethylacetate as the extracting solvent the solvent-vapour delivery tube (F) has to be isolated. A known volume of the phenolic stock solution was added to a volume of doubly distilled water in the sample container (A'). The water for the condensor was cooled by passing it through melting ice.

The boiling of the extracting solvent was started 10 minutes before the introduction of the sample. The sample flow was set by means of the teflon lined stopcock. When larger sample volumes were processed small portions of additional extracting solvent had to be added, through side arm H, to compensate the losses because of the solvents solubility in water. After the complete sample had passed the extraction chamber C, the circulation of the extracting solvent was continued for another 10 minutes. In this way the remaining organics were transferred to B. Of this extract 1 µl. was injected into the gas chromatograph.

The recovery being the ratio of the amount of a component present in the extract and the original sample or a representative duplicate was calculated from the ratio of the corresponding peak areas.

Between successive runs the apparatus was cleaned by rinsing with aceton and water and drying in an oven at 80–100 °C for about 2 hours.

Theory

In order to predict either the applicability of the continuous liquid extraction with the steam distillation-extraction device and/or the influence of process and component dependent factors, a theoretical model is derived. A schematical representation of volumes, flows and concentrations during the continuous liquid extraction process is depicted in Fig. 2.

To simplify the mathematical treatment, the extraction is proposed to be a process in a stationary state. The concentration in the sample delivered to the extraction compartment is constant and the concentration in the extracting

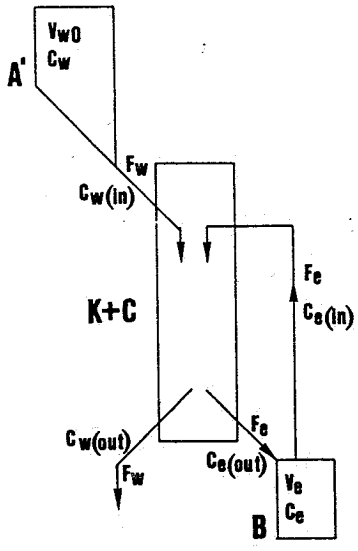

Fig. 2

Schematic representation of flows and concentrations during the continuous extraction process.

F = gas or liquid flow-rate, V = volume
C = mass concentration
w = water-sample e = extracting solvent
(in) denotes: to the extraction compartment
(out) denotes: out of the extraction compartment

solvent delivered to the condensor is assumed to be negligible (Ce(in) = 0), justifying a stationary treatment.

The process of continuous liquid extraction can be described by the following basic equations:

– the mass-balance over the extraction/separation compartment,

$$F_w \cdot C_{w(in)} = F_e \cdot C_{e(out)} + F_w \cdot C_{w(out)} \quad (1)$$

– the extraction coefficient,

$$K = \frac{C_{e(out)}}{C_{w(out)}} \quad (2)$$

– the mass-balance over the extracting solvent reservoir,

$$\frac{dC_e}{dt} = F_e \cdot C_{e(out)} \quad (3)$$

assuming thermodynamic equilibrium, ideal mixing in all phases, constant flows and volumes and Ce(in) to be negligible.

Combination of the eqs. (1) and (2) yields an expression for Ce(out). After substitution of this expression in eq. (3) and integration, the extracted amount can be expressed as:

$$C_e \cdot V_e = F_e \cdot C_{w(in)} \left\{ \frac{KF}{K+F} \right\} \cdot t \quad (4)$$

$$\text{with } F = \frac{F_w}{F_e}$$

The process can only last until the sample has been completely delivered to the extraction compartment, so at t = Vw, o/Fw the extraction process stops.

The recovery, defined as the ratio of the amount of the component in the extract and the amount originally present in the sample, can be expressed as:

$$\text{rec.} = \frac{C_e \cdot V_e}{C_{w(in)} \cdot V_{w,o}} = \frac{K}{K+F} \quad (5)$$

The recovery value is, theoretically, solely determined by the distribution constant (K) and the flow-ratio (F).

In case of a normal liquid-liquid extraction an identical relation can be derived:

$$\text{rec. (L.L)} = \frac{K}{K+V} \quad (6)$$

$$\text{with } V = \frac{V_w}{V_e} \text{ (the volume ratio)}$$

Obviously, equal recovery values are obtained for both techniques if the flow-ratio (F), for the continuous extraction, is equal to the volume ratio (V) for the normal extraction.

The concentration in the extract for both techniques can be expressed as:

$$C_e = C_w \cdot \frac{V_w}{V_e} \cdot \text{rec} \quad (7)$$

The volume ratio will normally be higher in case of a continuous extraction so enrichment of the extract can easily be obtained.

Results and Discussion

Equipment

In order to enlarge the contact time between the extracting solvent and the aqueous sample, the condensor of the extraction device was provided with a glass helix (c.f. Fig. 1). Comparative steam distillation-extraction experiments were performed to test this new condensor. Phenolic samples (50 ml; 200 ppb) were processed during 30 minutes according to the procedure given in reference [3]. The recoveries, typical values are given in Table I, indicate an improved extraction efficiency of about 20 % with the condensor provided with the glass helix.

Reproducibility and accuracy

Adsorption of phenolic substances on the boiling chips and the glass surfaces of the extraction apparatus was found to be negligible. Blank runs indicated the process to be very clean, no artifacts were seen.

The overall reproducibility of the continuous liquid extraction was determined. Both methylene chloride and ethylacetate were used as the extracting solvent. The concentration of the aqueous phenolic sample was 33 ppb per component. The sample volume was 30 ml. The total extraction time was 45–50 minutes.

The average recovery values and their corresponding relative standard deviations are given in Table II. M-, and p-cresol could not be separated under the chromatographic conditions used, so they are treated as one component. The overall

Table I. Recoveries of some phenols after steam distillation-extraction using different condensors.
Fw = 0.6 ml/min.; Fe = 1.1 ml/min.

Component	Recovery (%) old condensor	new condensor
phenol	33.2	44.7
m/p-cresol	46.2	66.7
3,5-dimethylphenol	56.5	82.1

Table II. Reproducibility of the continuous extraction.
RSD. = relative standard deviation (n = 5).

Component	Methylene chloride mean (exp)	RSD.	theory	Ethylacetate mean	RSD.
phenol	73.3	3.1	85	101.1	1.6
o-cresol	81.7	4.4	86	92.0	2.2
m/p-cresol	85.3	2.1	91	90.9	2.1
2,6-dimethylphenol	89.7	3.8	92	81.2	2.9
2,5-dimethylphenol	92.0	6.2	95	88.8	2.2
3,5-dimethylphenol	89.0	1.8	93	93.2	3.3
2,3-dimethylphenol	90.9	1.6	94	89.3	3.7
3,4-dimethylphenol	89.8	4.1	94	89.3	3.7
	mean	3.4		mean	2.6

Table III. Experimental extraction coefficients (K) measured for the system water/methylene chloride.

Component	K.
phenol	3.0
o-cresol	10.5
m/p-cresol	7.7
2,6-dimethylphenol	27.6
2,5-dimethylphenol	14.6
3,5-dimethylphenol	12.5
2,3-dimethylphenol	15.1
3,4-dimethylphenol	11.6

standard deviation includes errors due to sample preparation and the extraction process and was found to be in the order of 3 %(RSD), which indicates the extraction process to be very reproducible. The reproducibility of the GC analysis and the preparation of the reference mixtures was measured to be 4 %(RSD).

The extraction coefficients

According to the procedure described in the experimental section, the extraction coefficients of the phenolic compounds are determined using methylene chloride and ethylacetate as the extracting solvents. The results for methylene chloride are summarised in Table III.

The accuracy of the procedure, specially for large K-values (>5), is fairly low. When the difference in concentration between the reference solution and the extract is small, the calculated distribution constant becomes less accurate.

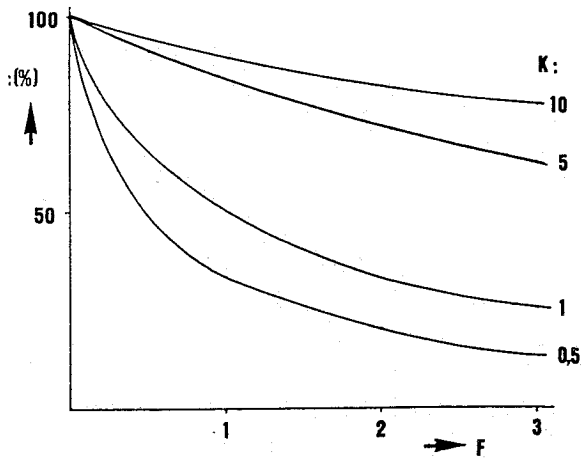

Fig. 3
The relation between the theoretically calculated recoveries and the flow-ratio (F) for different values of the water-solvent distribution constant, according to eq. (5).

The mean relative standard deviation for the values tabulated in Table III is about 20 %; for phenol a relative standard deviation of about 4 % was measured. Although the accuracy is limited, the given values serve well in the comparison between theory and experiment as can be seen in Table II. For the system water/ethylacetate no significant differences were measured between the reference solution and the extract for all test compounds. Hence, the extraction coefficients are assumed to be large.

Influence of the flow rate and the flow-ratio (F) on the recovery

The relation between the recovery and the flow-ratio, according to eq. (5), is plotted in Fig. 3 for different values of the extraction coefficient. At low flow ratios, e.g. a low water flow and/or a high flow of the extracting solvent, high recoveries will be obtained, even for polar compounds. Consequently, because the process time is inversely proportional to the water flow, long process times will be needed. At high flow ratios high recoveries will be obtained in a short time for apolar compounds which have relatively large extraction coefficients.

In practice the flows cannot be varied unlimited, but are controlled by the stability of the extraction process or the extraction time. A practical range for the flow-ratio is between 0.25 and 2.25, with the water flow varied between 0.5 and 4 ml./min.

The consistency of the theory with practice and the applicability of the continuous liquid extraction under several operating conditions will be demonstrated. Recoveries for some phenolic compounds using methylene chloride as the extracting solvent, measured at different flow rates and concentrations, are gathered in Table IV. The difference between calculated and experimental recoveries was only 10 %. The highest recoveries are obtained at the lowest flow-ratio. A mean recovery for all compounds of 94 % was measured in series 3, which is 97 % of the theoretical value. It can be concluded that at too high a water flow rate the system is far from equilibrium and too low recoveries rela-

Table IV. Recoveries for some phenolic compounds under different experimental conditions with methylene chloride as the extracting solvent.

Series	Sample-volume (ml)	Sample conc. (ppb)	Fw Fe F (ml/min)	Recovery (%) phenol	m/p-cresol	2,6-dmf
1	30	33	0.6 1.3 0.5	73.3	81.7	89.7
2	30	33	3.0 1.3 2.3	53.5	64.9	69.8
3	30	33	0.6 2.2 0.3	82.1	92.2	94.3
4	100	10	3.0 1.3 2.3	44.5	51.9	61.5
5	100	10	0.9 1.3 0.7	79.9	80.5	85.1

Table V. Effect of the water flow on the recovery (%) of some phenolic compounds using ethylacetate as the extracting solvent. Sample volume: 30 ml.; sample concentration: 33 ppb (w/v).

Component	Fw = 2.0 ml/min.	Fw = 1.0	Fw = 0.7	Fw = 0.5
phenol	78.6	82.4	101.1	100.2
o-cresol	74.1	85.0	92.0	101.4
m/p-cresol	75.4	85.0	90.9	98.9
2,6-dimethylphenol	76.9	77.2	91.2	100.5
2,5-dimethylphenol	75.9	82.1	88.8	99.4
3,5-dimethylphenol	75.7	80.0	93.2	102.5
2,3-dimethylphenol	72.6	78.6	87.2	97.8
3,4-dimethylphenol	72.7	80.4	89.3	99.0
mean	75.2	80.9	90.5	100.0
RSD.	2.7	3.0	6.2	1.5

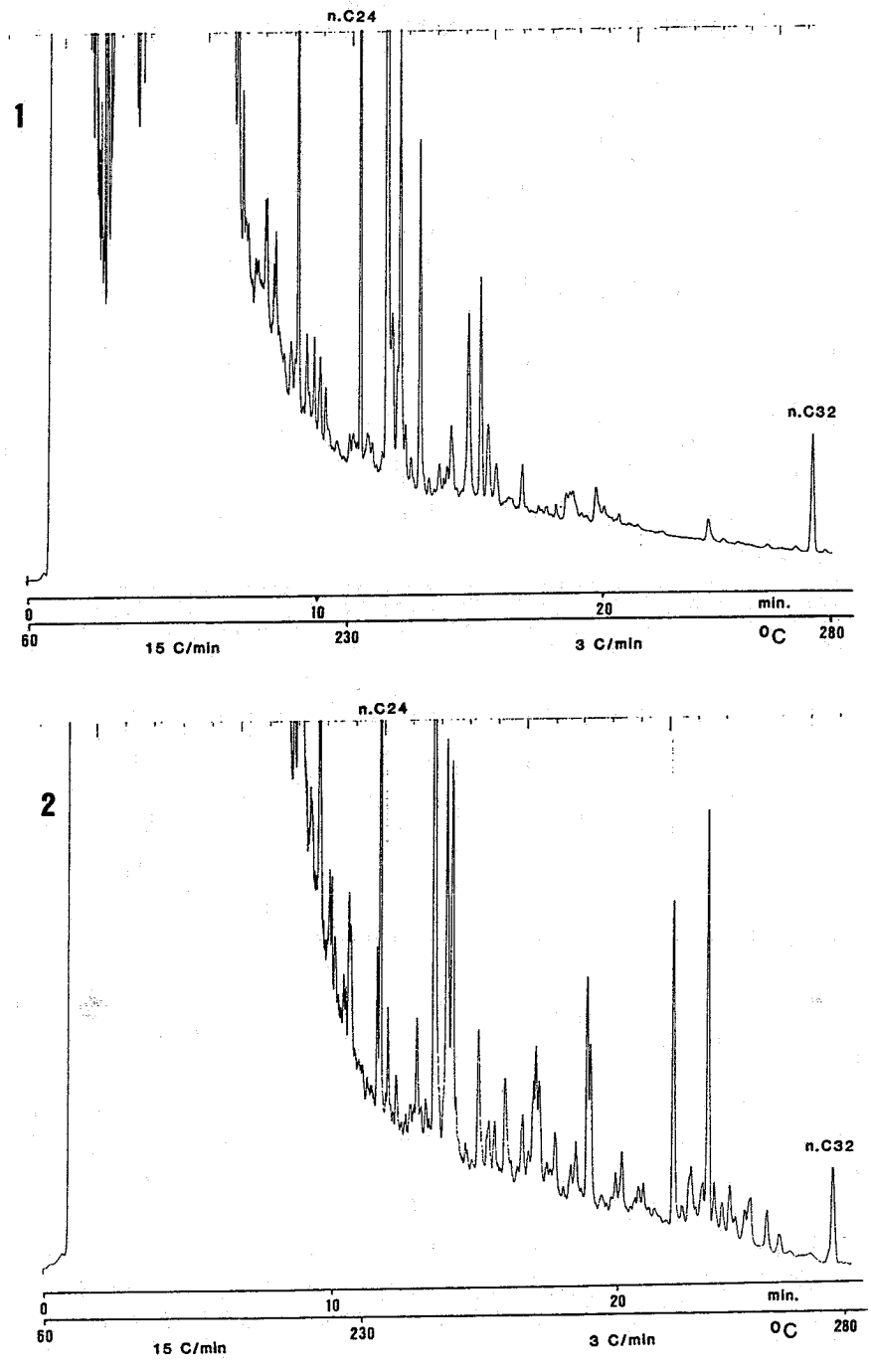

Fig. 4
Chromatograms of urine extracts after hydrolysis.
1: non derivatised.
2: after MO-TMS derivatisation

tive to the theory are measured. No significant concentration effect could be observed in the ppb and tens of ppb concentration range.

The effect of the water flow rate on phenolic compounds using ethylacetate as the extracting solvent is shown in Table V. The extraction coefficients for all the phenolic compounds in the system water/ethylacetate were found to be high, so that the recoveries for different phenols are not significantly different. Consequently the internal standard, with a high K-value, might be a phenolic compound and can be added directly to the sample. Again the lowest water flow rate gives the highest recoveries, about 100% for all compounds included in this study. At increasing water flow rate, with constant extracting solvent flow, the recovery decreases.

Applications

For the detection of the abuse of forbidden anabolic steroids ethyl acetate is often used for the extraction of non-conjugated urinary steroids [4, 5]. Extraction is performed after hydrolysis using Helix-Pomatia juice. A disadvantage of this procedure is the large volume of ethyl acetate (twice the urine volume of 10 to 30 ml.), which has to be evaporated to dryness prior to derivatisation. This evaporation step is time consuming while after continuous liquid extraction the final extract has a volume of about 1 ml., evaporation is completed in a much shorter period. A number of non-derivatised steroids can be analysed directly after extraction without further pretreatment prior to capillary GC-analysis.

Urine samples were hydrolysed prior to the continuous liquid extraction according to ref. [4]. After hydrolysis, 30 ml. urine was extracted with ethylacetate at a sample flow rate of 0.5 ml/min. A representative chromatogram of such a sample before and after derivatisation is shown in Fig. 4. The observed extraction efficiency was less than expected, either because of the proteins (Helix-Pomatia) in the sample or due to adsorption of free steroids in the non-deactivated extraction equipment. The results until now are rather promising. The procedure, however, has to be further optimised.

Conclusions

Both the theoretical calculations and the experimental results, which are in good agreement, show that continuous liquid extraction is a highly efficient and reproducible technique for the enrichment of organics from water samples. Although the detection limits largely depend on the sample volume, in any case ppb concentrations can be easily, and reliably analysed. The continuous liquid extraction as described extends the applicability of the steam distillation-extraction device for components with a low recovery in the steam distillation-extraction mode.

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