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SIMPLE AND DIRECT METHOD FOR QUANTITATIVE GAS CHROMATOGRAPHIC DETERMINATION OF DI(2-ETHYLHEXYL) PHTHALATE IN EDIBLE OILS

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SUMMARY

Di(2-ethylhexyl) phthalate (DEHP) has been extracted from edible oils by a column method and determined by gas chromatography. Only the minimum of apparatus and solvents are used to reduce the chances of contamination of the sample during the analysis. The lower limit of the concentration range examined is 40 ppm. Twenty samples collected from Danish consumers have been analyzed by the described method. In all the samples the concentration of DEPH has been found to be less than 40 ppm.

INTRODUCTION

For some decades, laboratories all over the world have found that phthalates used as plasticizers in polymers migrate to the surroundings. Today, phthalates are found almost everywhere. Modern laboratories, for example, are full of materials containing phthalates, *e.g.*, flooring, wall painting, packing, plastic and rubber tubings, rubber plugs and wires. From these materials the phthalates migrate into chemicals[1], solvents[1–5], glassware[1], filter-paper[1,6,7] and into the air[1,8].

Because of this contamination, any method for quantitative determination of di(2-ethylhexyl) phthalate (DEHP) in the ppm range requires that no polymers be used. This includes PTFE which often absorbs phthalates and later liberates them at random (authors' experience). It is also important that all apparatus is cleaned rapidly just before use. The reason for this is that phthalates present in the laboratory air will condense on the surface of the apparatus[1].

Preliminary experiments

Our preliminary experiments have shown that essential laboratory equipment such as rotary evaporators are potential sources of contamination. In several cases, in simulation experiments, higher concentrations of DEHP than added were found. The method of analysis and working procedure were examined and the potential sources of contamination were eliminated one by one until the rotary evaporator remained.

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To investigate this possibility, light petroleum, b.p. 30–50°, was purified following a procedure which will be described later. After the purification, the concentration of DEHP in this solvent was too small to be detected by direct injection of 2 μl into the gas chromatograph. A solution containing 50 ppm DEHP was made up in the purified light petroleum. An aliquot of this solution was mixed with internal standard, di(2-ethylhexyl) sebacate and used for gas chromatographic (GC) analysis.

A 5-ml sample of the DEHP solution was diluted with purified light petroleum to 30 ml and evaporated to dryness on the rotary evaporator. Several times during the evaporation, air was led into the rotary evaporator. After the evaporation the distillation flask was rinsed carefully with 5 ml of light petroleum, internal standard was added and the resulting solution was used for GC analysis.

In four cases out of 12 the increase in the DEHP concentration was so large that the DEHP would have been detectable by gas–liquid chromatography (GLC) if it had been originally present in the 30 ml before evaporation, showing that it was not just an increase in a non-detectable DEHP concentration in the purified solvent.

There are several ways of explaining this surprising result. It is possible that there are phthalates in the air sucked in from the laboratory during and after the evaporation[1,8]. Another possibility is that the phthalate is taken from the material of the tube (a *ca.* 50-cm tube of unknown polymeric material) that connects the air valve and the rotary evaporator, or perhaps the phthalate migrates from this and the other tubes on the rotary evaporator into the apparatus when it is not used and condense here on the internal surfaces, later to be sucked into the distillation flask with the air.

Because of this finding and the risk of contamination from the whole laboratory, the described method used no rotary evaporators or other relatively complicated glassware such as distillation and percolation equipment. Also, the evaporation of cleaned solvents was minimized.

EXPERIMENTAL

Purification of organic solvents

The phthalate contamination present in almost all organic solvents has caused severe problems in many laboratories[1–5]. Vessman and Rietz[2] have made unsuccessful attempts to purify solvents by distillation, treatment with acid, alkali or reducing agents, distillation over sodium and preparative GC. Our experiments with these methods have shown very similar results.

We did, however, succeed in eliminating the DEHP contamination from non-polar solvents in the following way. The contaminated non-polar solvent, methanol and sodium hydroxide in the ratio 30:30:1 were placed in an erlenmeyer flask having a ground-glass joint. The contents of the flask were then heated under reflux with magnetic stirring. After refluxing for 1–2 h no DEHP was detectable by injection of 2 μl of the non-polar phase into the gas chromatograph. The non-polar solvent was washed three times with water and dried over sodium sulphate. The sodium sulphate had been heated in an open oven at 500° for 3 h, partly to activate it and partly to eliminate any DEHP. Organic solvents which had been exposed to sodium sulphate for 24 h showed no evidence of contamination by DEHP (as demonstrated by GC analysis). This method was used for purifying light petroleum and toluene.

Toluene is soluble in both light petroleum and propylene carbonate and was

therefore used as a solvent for the internal standard. The propylene carbonate was purified by shaking three times with equal volumes of purified light petroleum. With the distribution ratio found (see later), this procedure effectively removes >99% of any DEHP present.

Triolein was chosen as a model for edible oils and a method described by Sampaolo *et al.*[9] was employed for purifying the triolein. The acetonitrile used in this method was purified in the same manner as the propylene carbonate.

After the removal of DEHP from propylene carbonate the latter was heated at 85° in an open flask for 24 h to eliminate any remaining extraction solvent.

Purification of the Celite

To remove any DEHP, the Celite was cleaned by washing with purified light petroleum, dried and heated at 500° for 12 h. The method was checked by adding a known amount of DEHP to the Celite. After the described purification, the Celite was subsequently employed in the column extraction method. The eluted propylene carbonate was collected in fractions of 1 ml, and 2 μl of each fraction were injected into the gas chromatograph. DEHP was not detectable in any of them.

Cleaning of glassware

All glassware was kept in chromic acid for at least 3 h before use. Glass wool was cleaned by heating it at 500° for 12 h. Only a few hours after the cleaning, when placed outside the oven, the glassware may be contaminated with detectable amounts of phthalates[1].

Gas chromatographic conditions

The experiments were carried out on a Hewlett-Packard Model 5711A gas chromatograph equipped with a flame ionization detector and glass column (200 × 0.4 cm I.D.) treated with dimethyldichlorosilane and packed with 5% OV-101 on acid-washed Chromosorb W (80–100 mesh). The column temperature was between 220 and 245°. Other details: injector block and detector temperatures, 300° and 350°, respectively; nitrogen flow-rate, 30 ml/min; injector membrane, Hewlett-Packard, Septa-low bleed.

Injection procedure

It is our experience that a 1-μl syringe with a wire inside the needle is very difficult to clean of DEHP. After as many as 50 washings with purified solvent, the syringe may not have "forgotten" the last sample with DEHP. The use of such a syringe is rejected in favour of a 5-μl syringe without a wire inside the needle, which does not cause such problems.

A 2-μl sample of each of the solvents was injected into the gas chromatograph with a 5-μl syringe. The same syringe was used to inject the samples. One microlitre of the sample was placed in front of a plug of cleaned solvent having a volume larger than that of the needle. The solvent plug prevents evaporation of components from the sample on the wall of the needle just after injection when the needle is still in the injector block. The sample and the cleaned solvent were separated by an air bubble (Fig. 1).

With GLC apparatus and the injection procedure described, it was possible to detect DEHP concentrations as low as 5 ppm (w/v) with reasonable accuracy.

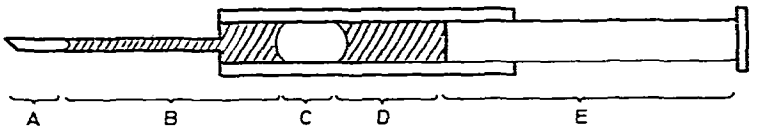

Fig. 1. Injector block. A = Air bubble to prevent contamination of the injector membrane; B = sample; C = air bubble; D = cleaned solvent; E = piston

When relating this to the lower limit (40 ppm) of the concentration range examined, this concentration can only be determined if the volume of the solvent containing the DEHP plus the volume of the internal standard solution does not exceed eight times the volume of the triolein. Due to the fact that all normal distillation equipment is avoided, and because of the unfavourable distribution ratio (see below) for DEHP between triolein and propylene carbonate (the preferred extraction agent), a simple batch extraction procedure is excluded.

Choice of extraction agent

It was confirmed by high-performance liquid chromatography that triglycerides are less soluble in propylene carbonate than in acetonitrile. Propylene carbonate was chosen as the extraction agent. Co-extracted triglycerides limit the use of gas chromatography since they cause considerable deterioration of the chromatographic column.

Triolein was chosen as a model for edible oils. The distribution ratio for DEHP between propylene carbonate and triolein was determined gas chromatographically.

$$D = \frac{\text{[DEHP] in triolein}}{\text{[DEHP] in propylene carbonate}} = 4.76$$

Column extraction

Owing to the unfavourable distribution ratio, a column extraction was used. To minimize the risk of contamination from the laboratory, the supporting material was coated in the column with the "contaminated" triolein.

Celite was chosen as the support material. At *ca.* 40% coating the Celite becomes sticky. To avoid this and to take into account random variations from batch to batch, the experiments were carried out with a 33% coating. The column was drawn out by heating a 30-cm glass tube (1.2 cm I.D., 1.4 cm O.D.) over a flame. After 3 h in chromic acid the column was washed with deionized water and dried. A plug of clean glass wool was placed at one end of the column which was then sealed by melting. The column was placed on a Mieler digital balance and triolein and purified light petroleum containing a known amount of DEHP were weighed directly into it. The liquids were mixed and the column was packed as desbribed in *Analysis procedure.*

Experiments were carried out using different amounts of stationary phase. To determine the elution of DEHP, the eluted propylene carbonate was collected in fractions of *ca.* 1 ml, internal standard was added and the resulting solution was used for GC analysis. The concentration of DEHP was determined from a calibration curve.

As expected, the experiments showed that, with increasing amounts of stationary phase, the DEHP was eluted in relatively smaller volumes of propylene carbonate, but

that this increased concentration results in a considerably increased analysis time. As a compromise between the analysis time and the precision, 2.66 g of stationary phase was chosen, *i.e.*, 2.00 g of Celite and 0.66 g of triolein. With this amount of stationary phase three series of experiments were done at a final DEHP concentration in the triolein of 240, 150 and 40 ppm, respectively. The lower limit of the concentration range examined (40 ppm) was chosen on the basis of the current Dutch law on the maximum limit for DEHP in foodstuffs. This limit has been proposed for adoption by the EEC. In all the experiments with 240 ppm the eluted liquid was collected in fractions of *ca.* 1 ml. The concentration of each fraction was determined. For the whole series there was none or only a trace amount of DEHP in the fifth fraction.

In subsequent experiments the first 5 ml were collected as one fraction, internal standard was added and the resulting solution was used for GC analysis. The data were treated statistically (see Results).

Analysis procedure

The solvents, Celite and equipment were cleaned and the column prepared as described above. Seven ml of light petroleum were placed on the column and 0.66 g of the edible oil were added. The column was placed on a vibrator. A glass tube containing 2 g of Celite was set at the top of the column, the Celite shaken down and mixed with the solution and the column then closed with a compact wad of glass wool and weighed.

After the suspended Celite had spread over the whole tube the column was placed in a horizontal position in an open oven at 50° with good ventilation until the Celite appeared to be dry. The temperature was raised to 100° and maintained until all the solvent had evaporated (this can be monitored by weighing).

The glass-wool wad was then pressed down through the tube with a glass rod until the stationary phase was coherent. The neck of the column was broken off and 10 ml of propylene carbonate were poured into the column. Approximately 5 ml of the added propylene carbonate were eluted and collected in a preweighed 10 ml flask. The volume of the propylene carbonate was calculated, a fraction removed, internal standard added and the mixture used for GC analysis.

RESULTS

The contamination of the edible oil can be determined with confidence limits of 95% in the following way.

(1) If the DEHP concentration is in the lower part of the range examined (40 ppm) the concentration determined will be 104 $\pm$ 15.5% of the contamination.

(2) If the concentration is in the upper part of the range examined (200 ppm) the concentration determined will be 98% $\pm$ 3.5% of the contamination.

Time of analysis

If the solvents and glassware are cleaned beforehand the analysis requires 10 min of manual work and 7 h waiting. The waiting period is partly for the drying (1–2 h) and partly for the elution. The latter can be carried out overnight since it stops when *ca.* 5 ml solvent has been eluted.

Application

The concentration of DEHP was always less than 40 ppm in the 20 edible oils collected from Danish consumers and analyzed by the method described.

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