Journal of Chromatography, 387 (1987) 509–514
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 19 139

Note

Analysis of di-(2-ethylhexyl)adipate plasticiser in foods by stable isotope dilution gas chromatography–mass spectrometry

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(First received August 19th, 1986; revised manuscript received October 8th, 1986)

Plasticisers are mainly organic esters of high boiling point, which are employed often in significant amounts in food contact materials to impart flexibility[1]. These compounds have been shown to migrate when the packaging material is in direct contact with foods, particularly those of a high fat content, and this may lead to contamination[2,3]. Assessment of this problem has necessitated monitoring of migration of plasticisers into foods, to enable an estimation to be made of the likely dietary intake of these contaminants.

As part of an initiative by the Ministry of Agriculture, Fisheries and Food (Working Party on Chemical Contaminants from Food Contact Materials) to identify overall plasticiser useage in the U.K. and to assess migration, quantitative methods of analysis have had to be developed for each of the plasticisers in a diversity of foodstuffs. Of the wide range of plasticisers, such as phthalates, phosphates, citrates and sebacates, that are employed in food contact materials, di-(2-ethylhexyl)-adipate (DEHA) is one of the most widely used in the U.K. in poly(vinyl chloride) film. This is used for retail packaging of foods, such as fresh meat, and in the form known as "cling-film" for wrapping foods in the home. In this note we report a gas chromatographic–mass spectrometric (GC–MS) method which was developed for DEHA to fulfill our requirements of high specificity, applicability to a diverse range of foodstuffs and high quantitative precision and accuracy.

A number of methods have been published for the estimation of plasticisers in foods; for example, DEHA has been determined in ground meat and fat samples after migration from plasticised film[4], using a lengthy method involving solvent extraction, saponification, steam distillation, ether extraction and finally quantification of the 2-ethylhexanol by GC with flame ionisation detection. The most extensive published studies on DEHA migration were into cheese and minced-meat[5], using a hexane–acetone lipid extraction of the food, size-exclusion chromatographic (SEC) clean-up and quantification by packed column GC with flame ionisation detection, enabling limits of detection to be achieved of between 10 to 30 mg/kg. Analogous methods have also been reported for the determination of the more general phthalate environmental contaminants in a variety of matrices such as eggs[6], fish[7] and other samples from the marine environment[8].

In the method reported in this paper, we have chosen (as in the case of many

0021-9673/87/$03.50

of the other published procedures) to use a size-exclusion clean-up of the DEHA from the co-extracted lipid, because of the simplicity of this approach, and the facility for automation. However, flame ionisation detection, which was employed elsewhere[4,5], is relatively non-specific and in view of the potential interferences from foods that have received comparatively little clean-up, capillary GC was employed with mass spectrometric multiple ion detection. This approach enables deuterated-DEHA to be incorporated as an internal standard, to correct for recovery throughout the assay and for it to be used for quantification.

EXPERIMENTAL

Materials

[2,2,5,5-$^2H_4$]Hexanedioic acid (98.8% deuterium incorporation) was obtained from MSD Isotopes (Cambrian Gases, Croydon, U.K.). DEHA was a commercial sample of plasticizer (Hexaplas) obtained from ICI, Plastics and Petrochemicals Division (Wilton, U.K.) and was shown by proton NMR not to contain more than 1% of detectable impurity.

Synthesis of deuterates internal standard

[2,2,5,5-$^2H_4$] Hexanedioic acid (1.98 g), 2-ethylhexanol (7.4 g) and boron trifluoride etherate (4.4 ml) in toluene were refluxed together for 1 h. After the addition of water, the product was extracted into ethyl acetate (60 ml), washed with water, sodium bicarbonate solution and dried with sodium sulphate. Excess alcohol was removed by evaporation, to yield 4.74 g of final product (yield 96%), the chemical purity of which was established as a single component by GC. Probe mass spectrometric analysis confirmed the identity of the product, with no discernible chemical impurities and an isotopic purity of $<0.3\%$ unlabelled DEHA.

Extraction and clean-up

Accurately weighed sub-samples of the homogenised foods (generally from 30 to 50 g) were blended with acetone–hexane (150 ml, 1:1) in an Ultra-Turax homogeniser. [$^2H_4$]DEHA internal standard (between 0.5 to 3.0 mg depending on the food type) was added from a 1 mg/ml solution in hexane, and the slurry set aside overnight to allow internal standard and analyte to equilibrate. The supernatant liquid was decanted from the residue, which was then re-extracted with a further two portions of solvent (75 ml each). The combined extracts were dried over sodium sulphate, evaporated to dryness, and the residue re-dissolved in dichloromethane–cyclohexane (1:1) to give a total volume of around 20 ml. Particulate matter was removed by low-speed centrifugation to give a clear solution for clean-up by SEC.

SEC clean-up

A fully automated SEC system as described elsewhere[9] was employed enabling auto-injection of samples and collection of fractions of interest in an unattended mode of operation. A 1 m × 25 mm I.D. glass column (Pharmacia, Uppsala, Sweden) containing an 80-cm bed of Biobeads S-X3 (Bio-Rad, Watford, U.K.) was operated at a flow-rate of 3.0 ml/min using dichloromethane–cyclohexane (1:1) solvent. An injection volume of 1.5 ml was employed and samples were diluted if necessary to

keep the fat content of the injected sample below 0.5 g.The elution time of the standard DEHA was established by GC analysis of trapped fractions, and the time window was thereafter used to collect the DEHA/[$^2H_4$] DEHA containing material from the fat extract. The column eluent was monitored at 254 nm and the samples were interspersed with solutions of phthalate esters, as UV-active retention time markers to ensure that the flow-rate was constant during unattended operation. The collected fraction containing DEHA was evaporated to dryness and transferred quantitatively to a small vial using acetone. The sample was then blown to dryness under nitrogen and stored at 5°C prior to GC–MS analysis.

GC–MS analysis

GC–MS analysis was carried out with a Carlo Erba 4160 GC instrument directly coupled to a VG 7070H mass spectrometer. The 25 m × 0.23 mm I.D. CP SIL 5CB fused silica column (Chrompack, U.K.) was operated at a helium carrier gas flow-rate of 1 ml/min, with split injection (1.5 μl, 30:1 split) at an isothermal column temperature of 220°C. The column outlet was connected directly to the mass spectrometer source held at 200°C, and the mass spectrometer was operated in an electron ionization mode at 70 eV with a 200 μA trap current. Multiple ion monitoring was carried out for *m/z* 129 (base peak for DEHA) and *m/z* 133 (analogous ion for [$^2H_4$]DEHA) with dwell times of 10 ms to give a cycle time of 250 ms. Quantification was on the basis of peak area ratio calibration curves for DEHA/[$^2H_4$]-DEHA at known ratios. This was checked by the analysis of foods of each type spiked with known amounts of DEHA.

RESULTS AND DISCUSSION

The extraction and SEC clean-up method reported was similar to that previously employed successfully in the automated clean-up for the analysis of organic phosphate contaminants in foods[10], and only differed from that reported elsewhere[5] for determining DEHA, in the present choice of solvent for the SEC stage. Dichloromethane–cyclohexane was preferred to ethyl acetate–toluene because of its greater volatility and thus its ease of removal from the final extract prior to GC–MS analysis. The fraction that was trapped from the SEC column eluted after the peak maxima for DEHA to give a recovery of about 50–60%. This low recovery was deliberate to minimise the extent of trapping of the tail of the partially resolved lipid peak that on GC analysis might otherwise have caused deterioration of the capillary GC column, and could potentially have given rise to interferences during GC–MS.

The electron ionization spectra of DEHA and [$^2H_4$]DEHA are shown in Fig. 1, from which it can be seen that the respective base peaks are at *m/z* 129 and *m/z* 133 and these were chosen for quantification. For a check on detection specificity in foodstuffs for which blank materials were not available the additional ions at *m/z* 147 and *m/z* 241 for DEHA were monitored, and the ion intensities shown to be in the ratio 1:1 at 10% of the *m/z* 129 base peak, as demonstrated for the authentic standard. We used capillary GC with split injection, rather than splitless or on-column to enable short isothermal chromatographic runs to be carried out, essential for the routine analysis of large numbers of sample extracts.

The method of analysis was applied to a very wide range of foods, such as

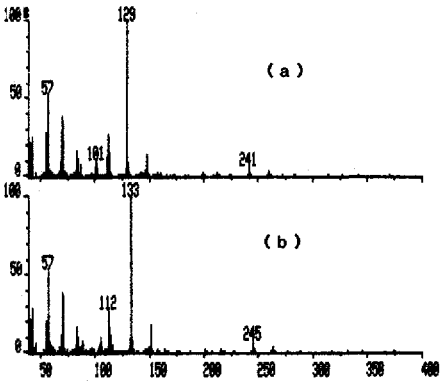

Fig. 1. Electron ionization mass spectra of (a) DEHA and (b) [$^2H_4$]DEHA internal standard. Spectra obtained by GC–MS at 70 eV ionization, scanning from m/z 500 to 25 at 1 s/decade. Source at 200°C with a trap current of 200 μA.

cheese, fresh and cooked meats, fish, sandwiches, cakes, fresh fruit and vegetables, and prepared cooked meals. In no instances was there any evidence of interference from co-extracted components in the determination of DEHA. Additionally, in experiments involving laboratory studies of migration from "cling-film", control foods were analysed that had not been in prior contact with the plastic and in all cases these foods did not contain detectable DEHA. The working limit of the method was chosen as 0.1 mg/kg, although the actual limit of detection was intrinsically far lower. In practice 0.1 mg/kg represented a level well below levels thought to be of any toxicological significance.

The use of the stable isotope internal standard compensated for losses throughout the procedure, enabling quantitative results to be reported without the need to correct for recovery, or the need to check constantly the repeatability of the extraction and clean-up procedure. This was particularly valuable in the ability of the method to tolerate comparatively low recoveries which avoided the problems of lipid interference, and in the ability to handle a diversity of food types, without the need in each instance to generate recovery data by spiking. For example, in the analysis of prepared meals which had been microwave cooked in contact with "cling-film" every sample was different from every other in composition, and without the stable isotope standardisation would have necessitated extensive method validation.

A typical calibration graph for the method is shown in Fig. 2, with a slight curvature in the calibration as is often the case with stable isotope standardisation. The calibration curves generated from different standards and analysed over a period of several weeks were found in each instance to be essentially superimposable. The inset in Fig. 2 shows the selected ions monitored for DEHA and internal standard, for both a calibration mixture and for a sample of cheese contaminated with DEHA by migration. The GC–MS selected ion chromatograms in all instances, as in the illustration in Fig. 2, showed smooth symmetrical peak shapes, against clean backgrounds and there was no difference in appearance between chromatograms for DEHA standards and for extracts of foods contaminated with DEHA. The precision of the method was checked by replicated analysis of homogenised sub-samples of

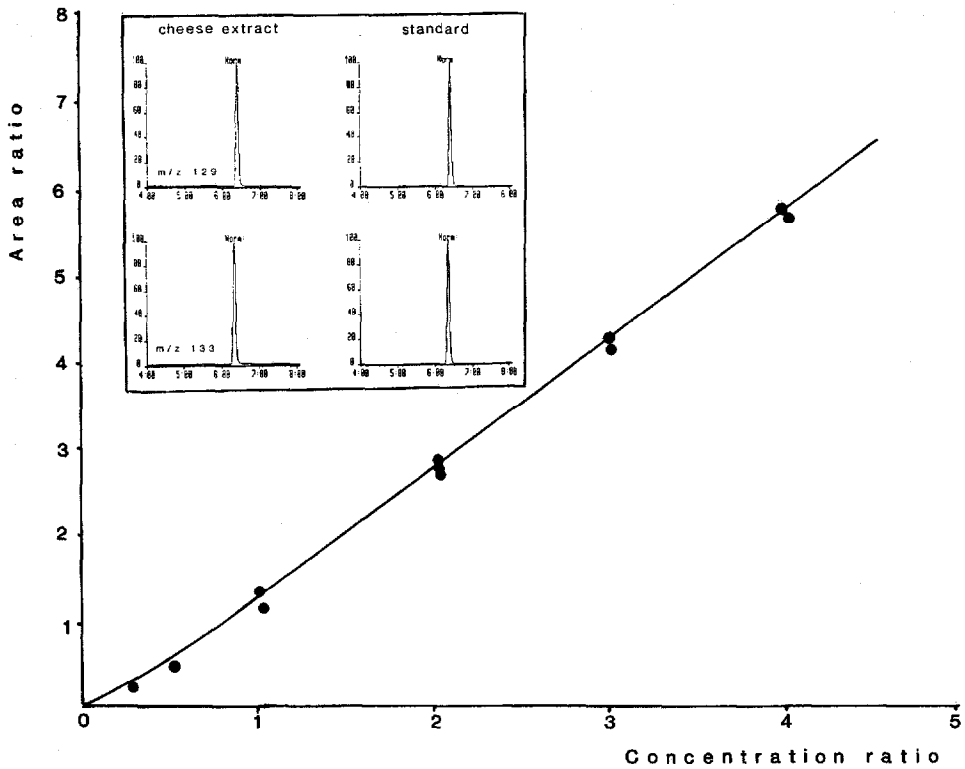

Fig. 2. Calibration graph for stable isotope dilution GC–MS analysis of DEHA. Plot of area ratio of *m/z* 129 to *m/z* 133 (internal standard) against concentration ratios. Inset illustrates selected ion monitoring traces for *m/z* 129 and *m/z* 133 for calibration standard and for contaminated cheese extract.

cheese contaminated at 5.4 mg/kg, and of cake contaminated at 78 mg/kg. The method was shown to have relative standard deviations of 0.9% (n = 10) and 3.2% (n = 5), respectively. The mean peak area ratios for DEHA spiked cheese samples was within 10% of the value expected for independantly prepared standards.

The method of analysis reported in this paper is currently being applied to the analysis of DEHA in a diversity of foods contaminated by migration during the domestic use of "cling-film", by microwave cooking using "cling-film", and from the retail packaging of foods in plasticised film; the results of these studies will be published elsewhere.

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