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Determination of Phthalates in Toys and Childcare Articles Made of PVC with HPLC and HPTLC

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Introduction

Phthalates are used as plasticizers to improve the flexibility or processability of plastic utensils, rubber items and other technical products such as paints. They are also used as fixatives for fragrances, as insect repellents and as solvents for pesticides. Bis-(2-ethylhexyl) phthalate (DEHP) is the most commonly used plasticizing agent for polyvinylchoride (PVC) with contents ranging between 10 and 40% in this plastic. It is therefore found everywhere in the environment. Despite its low acute toxicity, it is known to cause tumours in rats and mice exposed to high doses over a long period (1). Some phthalates also seem to influence the reproductivity of mammals (2). In 1998 the SCTEE (Scientific Committee for Toxicity, Ecotoxicity and the Environment) first established migration limits for six phthalates in soft PVC toys (3). In 1999 the European Commission banned childcare articles and toys made of soft PVC containing phthalates if these were intended to be put in the mouth by "under three year olds" (4). The initial approach based on migration limits was abandoned due to analytical problems and followed by a limit of 0.1% for the total content. Switzerland on the other hand has banned DEHP for these items since 1986 (5). A provisional limit referring to the total content of 10 mg/kg DEHP was later set to 0.1% for six phthalates in accordance with EC regulations. Reported methods concerning the determination of total content either used supercritical fluid, Soxhlet or Sonication extraction followed by identification and quantitation with gas chromatography (6–8). In this paper we would like to present our method for the determination of seven phthalates using tetrahydrofuran as an extractant and HPLC or HPTLC for identification and quantitation.

Method

Materials and instruments

Lab-shaker, Adolf Kühner AG, Switzerland; magnetic stirrer; rotary evaporator, Büchi R114 Switzerland; fluted filter, Schleicher & Schuell 602 EH; polyethylene syringes 1 ml; 0.45 µm Nylon filter, Gelman Acrodisc; HPLC system: quaternary low pressure mixing pump, Waters 600 MS; column oven, Waters Model 600; degasser, ERMA Tokyo ERC 3811; photodiode-array detector, Waters 996; Millennium chromatography software; column: Nucleosil C18,3 µm 120 Å, 250×4.0 mm, Macherey-Nagel; HPTLC system: CAMAG Reprostar 3 with Videoscan software; CAMAG Linomat IV sample applicator; CAMAG horizontal developing chamber for 10×10 cm plates; CAMAG TLC plate heater; HPTLC plates 10×10 cm RP-8_{W254S}, Merck.

Reagents

Tetrahydrofuran p.a., e.g. Merck 9731; methanol gradient grade, e.g. SDS 09337G16 for HPLC; acetonitrile gradient grade for HPLC, e.g. Lichrosolv, Merck 00030; demin. water Nanopur.

Reference materials

di-(2-ethylhexyl)-phthalate, *DEHP*, ≥97 % (Fluka 80032) CAS-Nr. 117-81-7,
di-butyl-phthalate, *DBP*, 99 % (Merck 800919) CAS-Nr. 84-74-2,
benzyl-butyl-phthalate, *BBP*, 98 % (Aldrich 30,850-1) CAS-Nr. 85-68-7,
di-n-octyl-phthalate, *DOP*, ≥98 % (Fluka 80153) CAS-Nr. 117-84-0,
di-iso-nonyl-phthalate, *DINP*, >99 % (Aldrich 37666-3) CAS-Nr. 28553-12-0,
di-iso-decyl-phthalate, *DIDP*, 99.8 % (Fluka 80135) CAS-Nr. 26761-40-0,
di-cyclohexyl-phthalate, *DCHP*, 96 % (Merck 800920) CAS-Nr. 84-61-7

Procedures

Calibration solutions for HPLC

Stock solutions

Prepare 100 ml solutions of 100 mg of each reference compound in acetonitrile (1 mg/ml). These solutions are stable for at least four weeks if stored at 4° C in the dark.

Calibration solutions

Pipette 5 ml of each stock solution into the same flask and dilute to 50 ml with acetonitrile (dilution 1). Pipette 25 ml of dilution 1 into a flask and dilute to 50 ml (dilution 2). Pipette 10 ml of dilution 1 into a flask and dilute to 50 ml (dilution 3).

Pipette 5 ml of dilution 1 into a flask and dilute to 50 ml (dilution 4). Pipette 1 ml of dilution 1 into a flask and dilute to 50 ml (dilution 5).

Calibration

Inject 10 µl of dilution 1 to 5. The corresponding amounts (depending on weighed-in quantity) are: dilution 1 (1 µg), dilution 2 (0.5 µg), dilution 3 (0.2 µg), dilution 4 (0.1 µg), dilution 5 (0.02 µg).

HPLC parameters and eluant

Temperature: 40° C, flow rate: 0.80 ml/min, detection wavelength: 225 nm, run time: 26 min, injection volume: 10 µl, DAD: Wavelength range: 200–320 nm, measuring time: 26 min, rate: 2.0 Hz, resolution: 1.2 nm (table 1).

Table 1
Peak retention time of phthalates

compound	*retention time in minutes*
DBP	4.5
BBP	5
DCHP	7.5
DEHP	14.1
DOP	15.1
DINP	highest peak at 18.6 (6 maxima)
DIDP	highest peak at 21.0 (3 maxima)

Calibration solutions for HPTLC

Prepare 100 ml solutions of 100 mg of DINP respectively DIDP in acetonitrile.

Solvent for HPTLC

Mix 25 ml of acetonitrile, 17.5 ml of tetrahydrofuran and 7.5 ml of water in a glass stoppered Erlenmeyer flask.

Setting of linomat IV

Plate width: 100 mm, start position: 8 mm, band: 5 mm, space: 4 mm, sec/µl: 15, volume: variable

Apply bands 7 mm from lower plate edge.

Application scheme for HPTLC

track 1: 1 µl each of DINP and DIDP solution (corresponds to 1 µg of each analyte)
track 2: 10 µl of sample extract
track 3: 2 µl each of DINP and DIDP solution
track 4: 10 µl of sample extract and 1 µl solution of the presumed phthalate
track 5: 3 µl each of DINP and DIDP solution

track 6: 10 µl of sample extract
track 7: 4 µl each of DINP and DIDP solution
track 8: 10 µl of sample extract and 1 µl solution of the presumed phthalate
track 9: 5 µl each of DINP and DIDP solution

Development of HPTLC plates

Fill developing chamber with solvent and condition plate for 15 min. Let solvent migrate for 50 mm. Dry for 5 min at 85° C with plate heater.

Documentation and quantitation

Record and quantitate HPTLC plates at 254 nm.

Sample preparation for HPLC and HPTLC

Weigh 1 g of sample in a 300 ml stoppered Erlenmeyer flask. Add 50 ml of tetrahydrofuran and stir about 15 min until the sample is dissolved. If the sample hasn't dissolved completely, place for another 15 min in an ultrasonic bath. Slowly add 150 ml of methanol while stirring. Depending on sample composition a precipitate might form. Store flask in an (explosion proof) refrigerator for 2 h. Filter suspension over a fluted filter and rinse filter with 50 ml methanol. Evaporate filtrate with a rotary evaporator at 40° C and 200 mbar and dry for 3 min at 70 mbar. For HPLC: Redissolve the oily residue with 50 ml acetonitrile. (For HPTLC: Redissolve the oily residue with 5 ml acetonitrile.) Store the suspension for 2 h in a refrigerator. Filter an aliquot through a 0.45 µm filter with a syringe. The filtrate is then ready for HPTLC or HPLC.

Evaluation of chromatograms (HPLC)

Calculate peak areas at 225 nm. Peaks are assigned according to retention time and UV spectrum (the UV spectra of the different phthalates are very similar) (fig. 1).

DINP and DIDP are isomere mixtures and give broad humps instead of discrete peaks with definite retention times. They therefore are easier to quantify with HPTLC.

Evaluation of chromatograms (HPTLC)

DINP and DIDP give dark bands at 254 nm. The rf-values are 0.24 (DINP) and 0.20 (DIDP).

Quality control

Samples should be stored at 4° C because phthalates tend to decompose. In case of positive samples, identification should be verified and recovery rates determined by repeating procedure with a sample aliquot spiked with the presumed phthalate. Even though phthalates are ubiquitously distributed in the environment and are

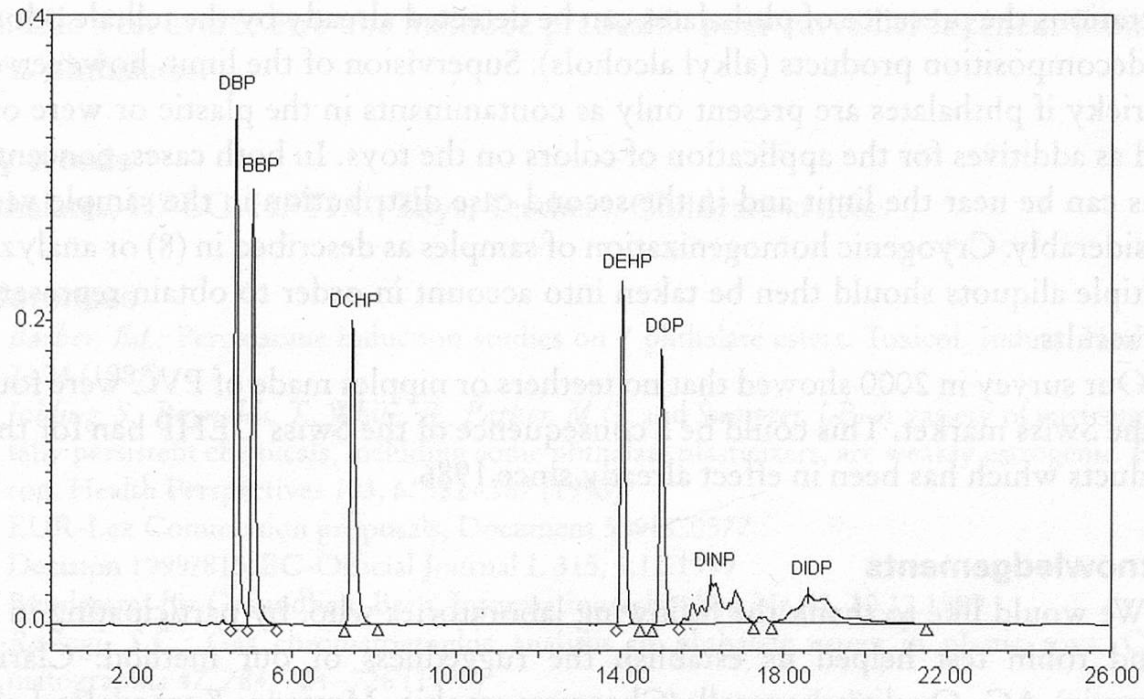

Figure 1 **HPLC run of phthalate standards**

known as laboratory contaminants, blank values are not a problem when supervising the limit of 0.1 %.

Results and discussion

With the HPLC procedure, phthalates can be directly determined in concentrations ranging from 0.01 % to 0.5 %. Quantitation with HPTLC can be directly applied between 0.05 % and 0.25 %. Samples with higher contents must be diluted prior to quantitation. Detection limits for DBP, BBP, DEHP and DOP are 5 mg/kg and for DINP and DIDP 0.025 %. The HPLC method was used in an interlaboratory test to verify, if it was adequate for supervising the current Swiss limit of 0.1 % phthalate. Four laboratories participated using the HPLC method and one laboratory used its own GC/MS method. Three samples were analysed of which sample 1 had no detectable amounts of phthalate, sample 2 contained about 27 % DEHP and sample 3 had about 24 % DINP. Only one laboratory used HPTLC to quantify DINP. All in all the results of the five laboratories were in good agreement. None of the laboratories detected any phthalates in sample 1. Interlaboratory relative standard deviation for sample 2 containing DEHP was 5 % and for sample 3 containing DINP was 4 %. Even though HPLC was not intended for quantifying DINP, results did not differ from those obtained with HPTLC. Recovery rates determined in our laboratory on a sample spiked with all seven phthalates lay between 88 % for DIDP and DINP (HPTLC method) and 97 to 101 % for DBP, BBP, DCHP, DEHP and DOP (HPLC method). When used as a plasticizer in PVC, phthalate concen-

trations are at least hundred times higher than the limit of 0.1 %. In such high concentrations the presence of phthalates can be detected already by the telltale odor of the decomposition products (alkyl alcohols). Supervision of the limit, however, can be tricky if phthalates are present only as contaminants in the plastic or were only used as additives for the application of colors on the toys. In both cases, concentrations can be near the limit and in the second case distribution in the sample varies considerably. Cryogenic homogenization of samples as described in (8) or analyzing multiple aliquots should then be taken into account in order to obtain representative results.

Our survey in 2000 showed that no teethers or nipples made of PVC were found on the Swiss market. This could be a consequence of the Swiss DEHP ban for these products which has been in effect already since 1986.

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Summary

A HPLC and HPTLC method for the determination of seven phthalates in childcare articles and toys made of PVC is presented. Extraction is performed by dissolving the PVC in tetrahydrofuran. Identification and quantitation is either performed by HPLC for phthalates giving discrete peaks or by HPTLC for DINP and DIDP. The method was used for an interlaboratory test and for a market survey and proved to be suitable for supervising the legal limit of 0.1 % phthalate.

Zusammenfassung

Es wird eine HPLC und eine HPTLC Methode beschrieben, mit der Phthalate in Spielzeugen, Beissringen und Saugern aus PVC bestimmt werden können. Die Extraktion wird durch Auflösen des PVC in Tetrahydrofuran erreicht. Die Identifikation und Quantifizierung wurde für Phthalate, welche diskrete Peaks ergaben, mit HPLC und für DINP und DIDP mit HPTLC durchgeführt. Die Methode wurde in einem Ringversuch getestet und für eine Marktkontrolle verwendet. Sie erwies sich als geeignet für die Überwachung der Limite von 0,1 % Phthalat.

Résumé

La méthode présentée permet de déterminer les phthalates dans les jouets et les sucettes en PVC. L'extraction se fait par dissolution du PVC dans du tétra-hydrofuranne. L'identification et la quantification sont faits par HPLC pour les phthalates donnant des pics discrets ou par HPTLC pour le DINP et le DIDP. La méthode a

été contrôlée par un test interlaboratoire et a été utilisée pour un contrôle du marché. Elle s'est avérée être une méthode praticable pour surveiller la teneur limite de 0,1 % phthalates.

Key words
Phthalates, HPLC, HPTLC, Toys, Teethers, Childcare articles

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