KNAUER

Application Note

► Determination of phthalates

Photo: crimex.de – duck without softeners

Category	Environmental analysis
Matrix	Plastics, PVC
Method	HPLC
Keywords	Phthalates, exposure, consumer products, softener, plasticizer, PVC
Analytes	Benzylbenzoate, butyl benzyl phthalate (BBP), dibutyl phthalate (DBP), dihexyl phthalate (DHP), di-(2-ethylhexyl) phthalate (DEHP), di-n-octyl phthalate (DNOP), di-isononyl phthalate (DINP), diisodecyl phthalate (DIDP)
ID	VEV0004N, 05/10

Summary

A reliable and robust method for the separation and determination of eight commonly used phthalates in consumer products is presented in this application note. The C18 alkyl stationary phase Eurospher II is applied to separate the phthalate mixture. Reduction of analysis time from about 35 to less than 22 minutes is achieved by employing 3 instead of 5 µm particles as the stationary phase. A binary low pressure gradient instrumentation is applied at a flow rate of 0.6 ml/min in combination with a 3 mm column ID and a UV-detector.

Introduction

Phthalates are in the majority of cases used as plasticizers in flexible polyvinylchloride products. The chemical industry produces according to the "European Council for Plasticisers and Intermediates" (ECPI) about 1 mill. tons every year only for western Europe. Although their vapor pressures are generally low, phthalates may occur including a vapor phase. Their general lipophilic character influences the leaching and environmental partitioning characteristics. [1]

Phthalates are evaporated from consumer products or find their way into the environment by abrasion from PVC particles. It becomes obvious that humans get into contact with these substances easily. Potential pathways of exposure are ingestion, inhalation, intravenous injection and skin absorption. Consumer products containing phthalates can result in human exposures through direct contact and use, by leaching into other products, or via general environmental contamination. Phthalates and their metabolites can be found in every human today, for example in urine or blood. [1,2]

Not at least caused by their adverse health effects, phthalates have to be monitored critically. In this work, eight of the most commonly used phthalates, namely Benzylbenzoate, butyl benzyl phthalate (BBP), dibutyl phthalate (DBP), dihexyl phthalate (DHP), di-(2-ethylhexyl) Phthalate (DEHP), di-n-octyl phthalate (DNOP), di-isononyl phthalate (DINP) and diisodecyl phthalate (DIDP) are separated. Their chemical structures are shown in figure 1.

The EU classified DEHP, DBP and BBP as toxic to reproduction and banned them especially from baby products. In many cases they can be replaced by DIDP and DINP for example, which are until now not regarded as toxic. Baby products are an exception, where these softeners are also forbidden for preventative reasons. DINP and DIDP are under suspicion for quickly spreading in the environment and accumulating in organisms. For this reason, their entry in the environment has to be inhibited. The German "Umwelt Bundesamt" suggests replacing all phthalate containing materials like flexible PVC little by little with phthalate free materials like polyethylene and polypropylene where it is possible. [2]

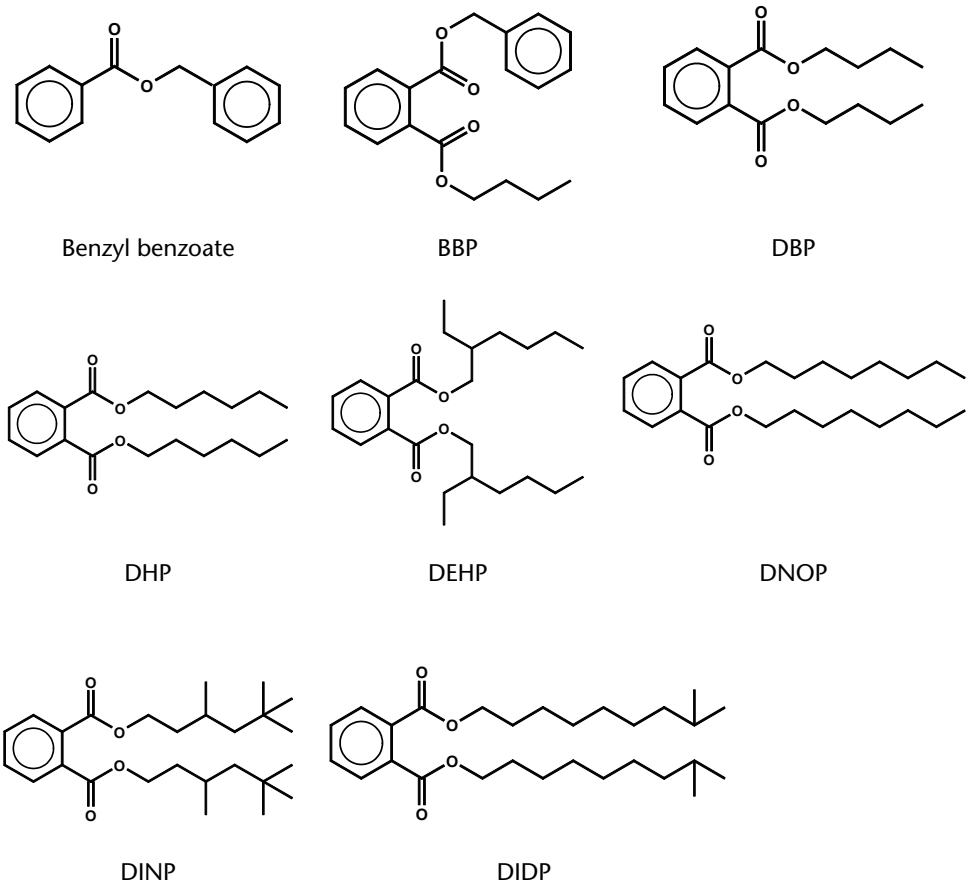

Fig. 1
Structures of analyzed Phthalates

Experimental sample preparation

According to the United States Consumer Product Safety Commission (CPSC) Test Method CPSC-CH-C1001-09.1, phthalates can be extracted from consumer products after comminution. An amount of 0.05 g of the crushed sample is collected in a glass vial and 5 ml of THF are added. The vial is shaken until the sample is dissolved what may take 30 min up to 2 h depending on the material. Polymers are precipitated using 10 ml of hexane or methanol in combination with cooling. When the polymers have settled, the solution is filtered through a 0.45 µm PTFE filter, evaporated and then diluted again with acetonitrile. After optionally adding an internal standard and dilution depending on the phthalate concentration the sample can be analyzed by HPLC. [3,4]

Experimental preparation of standard solution

In this work a stock solution was made by weighing out the single compounds, dissolving and mixing them in concentrations noted in table 1. Benzylbenzoate can act as an internal standard when samples are prepared. After dilution 1:10 with water/acetonitrile 15:85 (v/v) the standard solution is ready for analysis by HPLC.

Table 1
Concentration of Standard solution

Component	Concentration [mg/ml]
Benzylbenzoate	40.0
BBP	200.1
DBP	200.6
DHP	194.2
DEHP	206.0
DNOP	197.1
DINP	231.1
DIDP	211.5

Method parameters

Column	Eurospher II 100-3 C18 H, 250 x 3 mm		
Eluent A	Water/Acetonitrile 15:85 (v/v)		
Eluent B	Acetonitrile		
Gradient	**Time [min]**	**% A**	**% B**
	0.0	100	0
	3.0	100	0
	6.5	0	100
	19.5	0	100
Flow rate	0.6 ml/min		
Injection volume	2 µl standard		
Column temperature	30 °C		
System pressure	approx. 235 bar		
Detection	UV at 225 nm (5 Hz, 0.2 s)		
Analysis time	21 min		
Run time	45 min		

Results

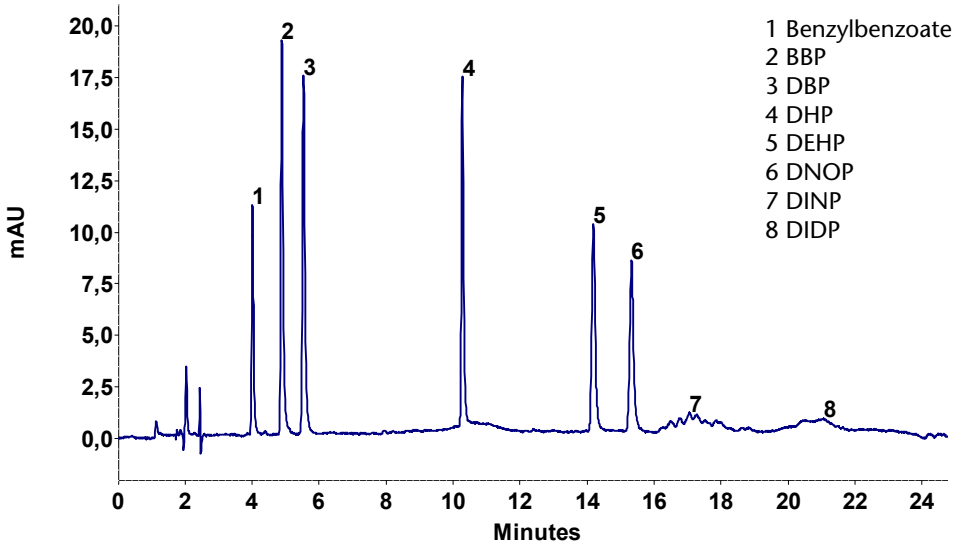

Fig. 2

Chromatogram of the phthalate standard solution (2 µl)

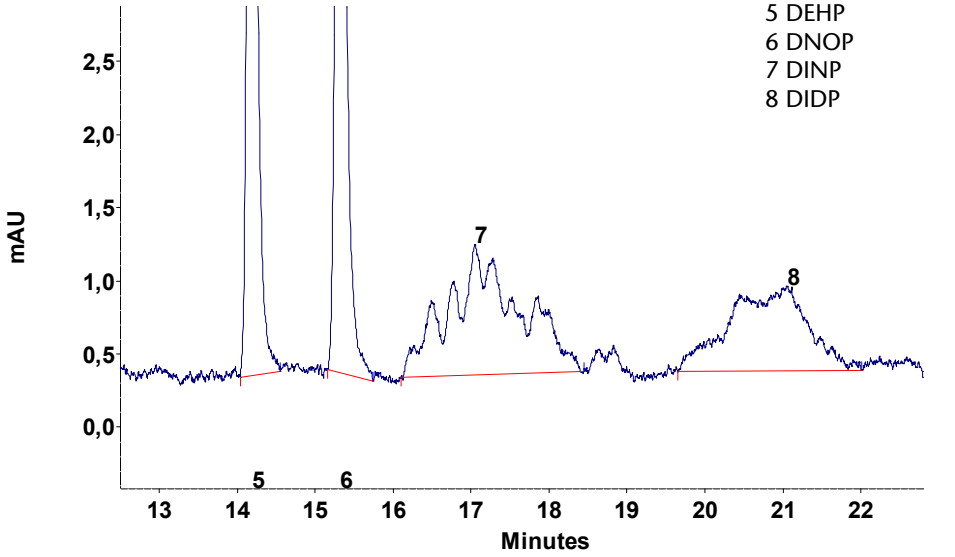

Fig. 3

Chromatogram zoomed in for Peaks 7 and 8

A chromatogram of the phthalate standard is shown in figure 2. All of the eight substances are baseline separated with resolution values in the range of 5 for the critical pair BBP and DBP, up to 38 for DHP. By optimizing the method parameters like flow rate and gradient steps, analysis time could be reduced from nearly 30 to 22 min.
The limit of detection (LOD) is 0.11 µg for the internal standard Benzylbenzoate and lies in the range of 0.32 up to 0.78 µg for the other phthalates apart from DINP and DIDP which have to be regarded separately. It is obvious that the peak shape for these two substances is not as sharp as for the other phthalates and it seams as if DINP and DIDP are not eluting in one but in a sum of various peaks. The reason for these phenomena is the quality of the standard. Regarding the chemical structures in figure 1, it is noticeable that DINP and DIDP may occur in different isomeric forms. The quality of a single compound is normally described as "mixture of isomers" by the chemical industry, which can not easily produce DINP and DIDP free of isomeric forms. In this work, the sum of peaks is regarded as one for the evaluation as it is shown in figure 3. This results in a relatively high LOD of 7.8 µg for DINP and 10.9 µg for DIDP.

Conclusion

This application note describes a fast and robust method for the determination of eight phthalates that are commonly used by the industry. An easy separation in less than 22 minutes becomes possible by applying the Knauer Smartline HPLC System. The 3 mm inner diameter of the chosen Eurospher II column results in an eluent saving of 77 % compared to a conventional 4 mm column ID. Accessorily there is the possibility to use a shorter column if the separation of BBP and DBP is not of interest, because all other substances are separated with higher resolution values. Shortening the column would lead to a faster analysis and therewith to less usage of the mobile phase.

References

1 T. Schettler. Human exposure to phthalates via consumer products. International Journal of Andrology, 2006 (29), p. 134 - 139
2 Umwelt Bundesamt. Phtalate – Die Nützlichen Weichmacher mit den unerwünschten Eigenschaften. www.umweltbundesamt.de, 2007
3 United States Consumer Product Safety Commission (CPSC). Test Method: CPSC CH-C1001-09.1 Standard Operating Procedure for Determination of Phthalates. March 3, 2009. www.cpsc.gov/about/cpsia/phthalatesop.pdf
4 Dr. Urs Hauri, Kantonales Laboratorium Basel Stadt. Duschgele in Weich-PVC-Verpackungen / Phthalate und deren Retention auf der Haut. 31.12.2008 www.kantonslabor- bs.ch/files/berichte/Duschgel08_2.pdf

Physical properties of the recommended column

Based on an ultra pure silica gel, Eurospher II is a high performance column material for analytical, semi preparative and process-scale applications. Eurospher II features very narrow particle and pore size distributions, as well as outstanding mechanical stability. Eurospher II silica gel is perfectly suited to take on routine analyses as well as the most ambitious chromatography tasks.
Eurospher II C18 H is the recommended alternative for Kromasil 100 C18 columns. It works well for acidic, basic and neutral analytes in reversed phase mode with extended pH range.

Stationary phase	Eurospher II 100-3 C18 H
USP code	L1
Pore size	100 Å
Particle size	3 µm
Form	spherical
Surface area	320 +/- 20 m^2/g
% C	17
Endcapping	Yes
Dimensions	250 x 3 mm
Order number	25CE185E2G

Recommended instrumentation

The analysis was performed on the KNAUER low pressure gradient Smartline System, equipped with a Smartline Pump 1000 and the Smartline Manager 5000 with degasser, Autosampler 3950, Column Oven and UV-Detector 2600.

Description	Order No.
Smartline Pump 1000, incl. 10 ml pump head	A50303
Smartline Manager 5000 with degasser	A5316
Autosampler 3950	A5005
Smartline Column Oven 4050	A5300
Smartline UV Detector 2600	A5200
UV cell (10 mm), analytical version	A4061
ChromGate software	A1493

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