Short communication

Characterization of phthalate plasticizers by HPLC/thermospray mass spectrometry

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1. Introduction

Di-alkyl phthalate esters are frequently used as plasticizers in tubing, containers, label adhesives, and a wide array of materials that come into contact with pharmaceutical products or physiological fluid samples. The di-alkyl phthalate esters are extremely lipophilic and they are observed at very long retention times on most reversed-phase HPLC systems. Because of the aqueous nature of most samples, the esters are often partially hydrolyzed to the mono-alkyl phthalate esters which are observed at much shorter retention times. While there have been a number of reports on GC/MS methods for the characterization of di-alkyl phthalate esters, there have been very few LC/MS studies on the di-esters, and no LC/MS accounts of the mono-alkyl phthalate esters. As part of a study on environmental pollutants, several di-alkyl phthalates were characterized using a moving-belt type LC/MS system [1]. Using a thermospray mass spectrometry system, di-methyl and di-ethyl phthalate were detected in environmental samples by LC/MS [2], but no attempt was made to identify any mono-alkyl phthalate esters that may have been present.

The primary objective of the present study was to develop a thermospray LC/MS method for the detection and characterization of both the mono- and di-alkyl phthalates. While there are over 40 commercially available di-alkyl phthalates, six of the most commonly used di-alkyl phthalates and the six corresponding mono-alkyl esters were selected for the present study.
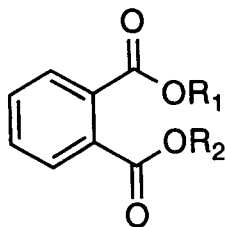
2. Experimental

The authentic reference standards of the phthalate di-esters were all obtained from Supelco (Bellefonte, PA; dimethyl phthalate, cat. no. 4-8562; diethyl phthalate, cat. no. 4-8561; di-*n*-butyl phthalate, cat. no. 4-8559; benzyl butyl phthalate, cat. no. 4-8558; bis(2-ethylhexyl) phthalate, cat. no. 4-8557; and di-*n*-octyl phthalate, cat. no. 4-8560). The test samples were prepared as 1 mg ml^{-1} solutions in acetonitrile. To prepare samples of the mono-esters, 1.0 ml of a 1 mg ml^{-1} acetonitrile solution of the di-alkyl esters was mixed

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Table 1
Reversed-phase HPLC retention and thermospray mass spectral characteristics of mono- and di-alkyl phthalate esters

R_1	R_2	t_r (min)	M+H	M+NH$_4$	M+1−HOR$_2$
H	H	1.5	167(65%)	184(100%)	149(4%)
H	CH$_3$	5.5	181(100%)	198(30%)	149(3%)
H	CH$_2$CH$_3$	8.2	195(100%)	212(10%)	149(4%)
H	CH$_2$CH$_2$CH$_2$CH$_3$	14.7	223(100%)	240(6%)	149(3%)
H	Benzyl	15.3	257(100%)	274(30%)	149(2%)
CH$_3$	CH$_3$	15.4	195(100%)	212(5%)	163(10%)
CH$_2$CH$_3$	CH$_2$CH$_3$	18.8	223(100%)	240(2%)	177(6%)
H	2-Ethylhexyl	21.5	279(100%)	296(4%)	149(3%)
H	*n*-Octyl	22.0	279(100%)	296(4%)	149(3%)
CH$_2$CH$_2$CH$_2$CH$_3$	Benzyl	23.5	313(100%)	330(14%)	205(3%)
CH$_2$CH$_2$CH$_2$CH$_3$	CH$_2$CH$_2$CH$_2$CH$_3$	23.8	279(100%)	<2%	205(2%)
2-Ethylhexyl	2-Ethylhexyl	28.0	391(100%)	<2%	261(3%)
n-Octyl	*n*-Octyl	28.3	391(100%)	<2%	261(5%)

[a] *m/z* value and relative intensity.

with 15 mg of Na$_2$CO$_3$ and 1.0 ml of water, and then warmed at approximately 60°C for 3 h, yielding approximately 50% conversion of the di-alkyl esters to the corresponding mono-alkyl phthalates.

The LC/MS studies were conducted using a Vestec Model 201B thermospray mass spectrometer that was interfaced with a Waters Associates Model 600MS HPLC system and a Waters Model 486MS ultraviolet detector (277 nm) plumbed in series with the mass spectrometer. The following mass spectrometer and HPLC conditions were utilized:

Mass spectrometer source temperature:	265°C
Thermospray vaporizer tip temperature:	218°C (with gradient compensation of 950)
Mass spectrometer operation mode:	positive ions, filament turned on

HPLC column:	Waters Symmetry C-8 (5μm), 3.9 mm × 150 mm, 1.0 ml min^{-1} flow rate
HPLC solvent A:	0.1 M ammonium acetate in 10:90 (v/v) methanol:water (no pH adjustment)
HPLC solvent B:	0.1 M ammonium acetate in methanol
HPLC linear A,B gradient:	0% to 100% B over 25 min, hold for additional 5 min.

3. Results and discussion

It was found that all of the mono- and di-alkyl esters gave strong (M + H)$^+$ pseudomolecular

Table 2
Reversed-phase HPLC retention and Hansch lipophilicity characteristics of mono- and di-alkyl phthalate esters

R_1	R_2	t_r (min)	Hansch π values $R_1 + R_2$	
H	CH_3	5.5		0.56
H	CH_2CH_3	8.2		1.02
H	$CH_2CH_2CH_2CH_3$	14.7		2.05
H	Benzyl	15.3		2.01
CH_3	CH_3	15.4	1.12	
CH_2CH_3	CH_2CH_3	18.8	2.04	
H	2-Ethylhexyl	21.5		3.85
H	n-Octyl	22.0		4.05
$CH_2CH_2CH_2CH_3$	Benzyl	23.5	4.06	
$CH_2CH_2CH_2CH_3$	$CH_2CH_2CH_2CH_3$	23.8	4.10	
2-Ethylhexyl	2-Ethylhexyl	28.0	7.70	
n-Octyl	n-Octyl	28.3	8.10	

ions (the base peak of the spectrum in most cases, Table 1). Because of the ammonium acetate used in the thermospray ionization process, most of the compounds also exhibited a moderately strong $(M + NH_4)^+$ ion. For thermospray mass spectrometry, as the percentage of organic modifier in the mobile phase increases, the intensity of the $(M + NH_4)^+$ ion relative to that of the $(M + H)^+$ ion will generally decrease. Thus with a gradient HPLC system as used in the present study, one would expect the relative intensity of the $(M + NH_4)^+$ ion of the phthalate esters to decrease as the retention time of the specific homologue increased because of the increased organic modifier in the mobil phase.

With the exception of the strong $(M + H)^+$ and $(M + HN_4)^+$ ions, the thermospray mass spectra of the mono- and di-esters showed very few other peaks and these were of low intensity (Table 1). As a subclass of compounds, all of the mono-esters were found to exhibit a small m/z 149 spectral line that corresponds to the loss of the remaining alkyl group. In contrast, all of the di-esters were found not to exhibit a m/z 149 peak, but the corresponding $(M + 1 - HOR_2)^+$ spectral line was detected and the specific m/z value was characteristic of the specific homologue. Both dimethyl phthalate and mono-ethyl phthalate exhibited m/z 195 $(M + H)^+$ ions as the base peak

in the spectrum, but dimethyl phthalate could be uniquely characterized by its m/z 163 spectral line and mono-ethyl phthalate could be uniquely characterized by its m/z 149 spectral line (Table 1). Using the same strategy, diethyl phthalate was distinguished from mono-butyl phthalate and dibutyl phthalate was differentiated from mono-octyl phthalate.

A sample containing a mixture of the mono- and di-alkyl esters was examined using selected ion monitoring (SIM) at m/z 149. Using this SIM method, all of the mono-esters were easily detected as individual chromatographic peaks, while none of the di-alkyl esters produced a m/z 149 response. Thus SIM (or reconstructed ion chromatograms as in the present study) might be useful for detecting this subclass of compounds, but the relatively low intensity of the m/z 149 peak might reduce the overall sensitivity of the method. Using thermospray mass spectrometry it has been generally observed that an increase in the source temperature will yield more extensive fragmentation in the mass spectrum. Thus one might be able to increase the sensitivity of the m/z 149 SIM by using a higher source block temperature.
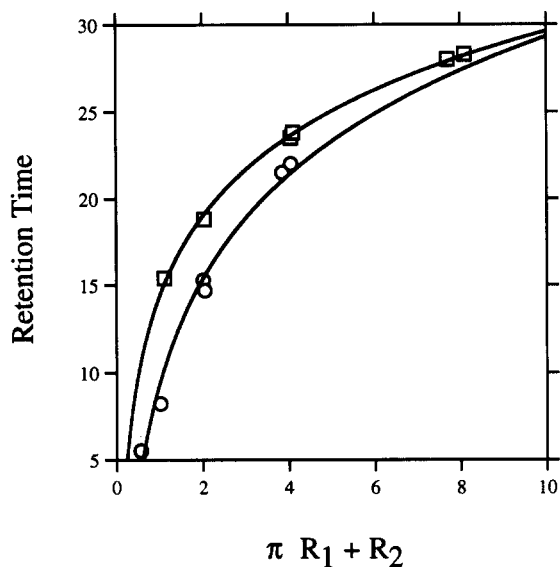

Fig. 1. Reversed-phase retention times of mono- (○) and di-alkyl (□) esters of phthalate acid as a function of the calculated π values of the substituents.

The retention times of the mono- and di-esters were also found to be correlated with the lipophilicity of homologues (Table 2, Fig. 1). The lipophilicities of the R substituents were calculated [3] using the Hansch-type constants (π values) and it was found that for both series of homologues, the retention time was found to increase exponentially (Fig. 1). For the mono-ester series, all of the homologues contained an ionized carboxylic acid group that would markedly decrease the lipophilicity of this sub-class. Thus for specific mono-esters and di-esters having the same π-value for the R group(s), one would expect the mono-ester to have the shorter retention time. For these reasons, the mono- and di-ester series each form an exponential t_r versus π series with a displacement in the two curves corresponding to the ionization of the carboxyl group.

For either linear or branched aliphatic phthalate esters, one could calculate the number of methylene groups (N) in the side-chains using the formula below where "X" was the observed $M + 1$ pseudomolecular ion. Thus for compounds that were not specifically a part of the present study, one could use the calculated value of "N" to suggest the total number of methylenes of R_1 and R_2 of a tentatively proposed structure.

$$N = \frac{(X - 167)}{14}$$

Of the above candidate structures, the mono-alkyl esters could be uniquely characterized by the presense of the m/z 149 spectral line, while the di-alkyl phthalates could be characterized by the appropriate $(M + 1 - HOR_2)^+$ spectral line. Due to the presence of the ionized carboxyl group, the mono-alkyl phthalate would be expected to have a much shorter retention time than the non-ionized di-alkyl ester with the same total number of methylenes. Using the calculated π value for the candidate structure, the estimated time (Fig. 1) should also be consistent with the experimentally observed value.

References

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[2] T.A. Bellar and W.L. Budde, Anal. Chem., 60 (1988) 2076–2083.
[3] Y.C. Martin, in Quantitative Drug Design, Medicinal Research Series Vol. 8, M. Dekker, New York, 1978.