

THE ANALYSIS OF PHTHALATE MONOESTERS BY HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY (HILIC)

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Introduction

Phthalate esters have been widely used for plasticizer of poly vinyl chloride (PVCs) and solvents in industrial and consumer products, therefore they are ubiquitously present in both indoor and outdoor environment. However, the effects of phthalate esters and their metabolites to reproductive function and allergic reaction are pointed out^{1,2}. Human ingests and inhales them via food, indoor air, indoor dust, outdoor air, water and soil and metabolize it to monoesters and their glucuronide. Total phthalate monoesters in urine can be used as a biomarker of representing exposure level of phthalate esters, and therefore have been analyzed in many epidemiological studies.

For the analysis of phthalate monoesters, tandem mass spectrometry (MS/MS) equipped with reversed phase (RP) LC has been used, while for urine sample preparation, the RP solid extraction is usual choice. However, this combination of same RP principle in clean-up and separation stage may not be efficient enough to remove interferences in urine matrices. Hydrophilic interaction liquid chromatography (HILIC) is an alternative chromatographic technique to effectively separate hydrophilic and small polar compounds on polar stationary phases. HILIC usually uses aqueous-organic solvent mobile phase with a high content of organic solvent. As the ionization efficiency of compounds tends to be higher in solvent of higher organic content, HILIC has occasionally been providing higher sensitivity than RP system. The application has rapidly increased in the past decade³. In contrast to RP, however, there are wide varieties of columns developed for HILIC mode, and selection of optimum column for phthalate monoesters is not obvious. In this study we compared the performance of several HILIC columns together with RP system for phthalate monoesters' analysis.

Materials and methods

Eight phthalate monoesters, i.e., monomethylphthalate (MMP), monoethylphthalate (MEP), mono-n-butylphthalate (MnBP), monobutylbenzylphthalate (MBzP), mono-n-octyl phthalate (MOP), monoisononylphthalate (MNP), mono-(2-ethylhexyl) phthalate (MEHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), were analyzed in this study. A hybrid triple quadrupole/linear ion trap mass spectrometer (4000QTrap, AB SCIEX) equipped with an Agilent 1200 series HPLC system (Agilent Technologies, CA) was used. The condition of MS/MS is described in Table 1 and the monitor ions of native and internal standard are given in Table 2. The first choice of mobile phase was 10 mM ammonium acetate for aqueous mobile phase and acetonitrile for organic mobile phase. We also used formic acid, ammonium formate and acetic acid as an alternative choice for aqueous phase. The sample injection volume was 5 μ l (acetonitrile) and the flow rate was 0.2 ml/min.

Table 1 Condition of MS/MS

Condition	
Ionization	ESI
Polarity	Negative
Ion spray voltage (V)	-4500
Nebulizer (psi)	50
Trubo gas (psi)	80
Temperature (°C)	600

Table 2 Monitored ion of native and internal standard

	Native	¹³ C-labeled	DP	CE
MMP	179/77	183/79	-50	-28
MEP	193/77	197/79	-45	-24
MnBP	221/77	225/79	-50	-28
MBzP	255/183	259/186	-60	-16
MOP	277/125	281/127	-70	-28
MNP	291/121	295/124	-65	-22
MEHP	277/134	281/137	-55	-22
MEHHP	293/121	297/124	-50	-26

DP: Declustering potential, CE: Collision energy

Seven different types of HILIC columns, i.e., PC HILIC (SHISEIDO, 2.0×150 mm, particle size 3 μm), ZIC HILIC (Merck, 2.1×150 mm, particle size 3.5 μm), Atlantis HILIC silica (Waters, 2.1×150 mm, particle size 3 μm), XBridge HILIC (Waters, 2.1×150 mm, particle size 3.5 μm), XBridge-Amide (Waters, 2.1×150 mm, particle size 3.5 μm), Cosmosil HILIC (Nacalai, 2.0×150 mm, particle size 5 μm), Shodex NH₂P40 (Showa-denko, 2.0×250 mm, particle size 5 μm) were tested. PC HILIC and ZIC HILIC are zwitterionic silica columns, Atlantis HILIC silica is bare silica column, XBridge HILIC is bare ethylene bridged hybrid (BEH) column, XBridge Amide is amide-bonded BEH column, Cosmosil HILIC is triazole-bonded silica column, and Shodex NH₂P40 is polymer-based amino column. For comparison Inertsil ODS-4 (GL science, 2.1×150mm, particle size 3μm) was used as RP column.

Results and discussion

The chromatogram of phthalate monoesters analyzed by RP column (a) and HILIC columns (b)-(h) are shown in Figure 1. The conditions of mobile phase were given in each figure.

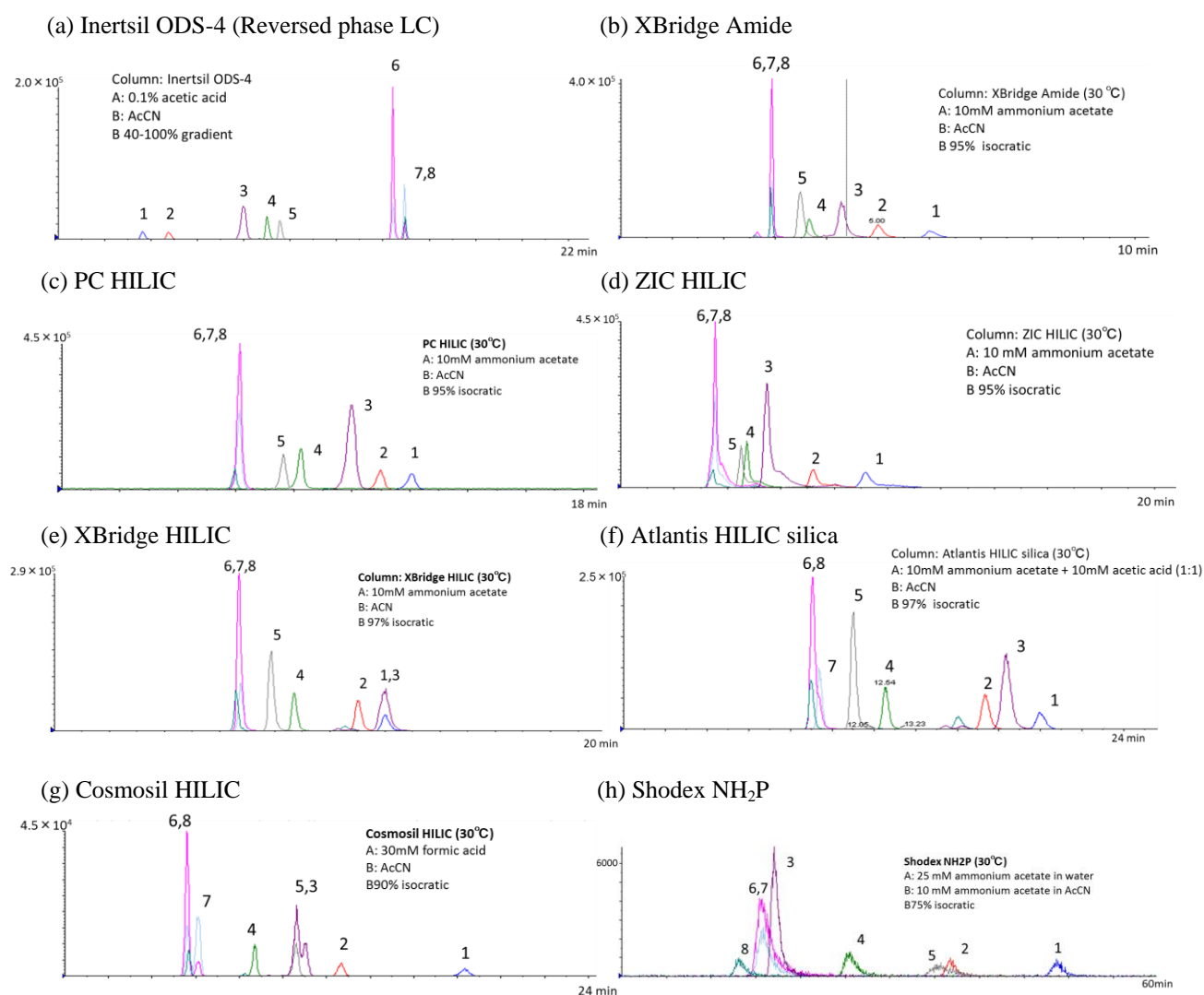


Figure 1. Chromatogram of phthalate monoesters analyzed by RP column and HILIC columns
 1: MMP, 2: MEP, 3: MEHHP, 4: MnBP, 5: MBzP, 6: MEHP, 7: MOP, 8: MNP

As expected, elution orders of phthalate monoesters from HILIC columns are generally in a reverse order to that of RP column, i.e., the most hydrophobic compounds MNP, MOP and MEHP, eluted first while the least hydrophobic MMP eluted last. There are, however, notable differences between HILIC columns as for the peak shape, intensity, and detailed elution order in some compounds. For example, MEHHP eluted between MEP and MMP from the Atlantis HILIC silica, while it eluted between MEHP and MBP from the Shodex NH₂P. The HILIC columns examined in the present study were divided into 4 groups; 1) amide, 2) zwitterion, 3) unmodified, 4) positively-charged. Characteristics of each group are summarized below;

1) XBridge Amide in the first group showed weaker interaction with phthalate monoesters than the other types. Although the peak shape of the first eluting group was sharp with enough sensitivity (Fig.1b), those with longer retention times tend to give broader and smaller peaks. The situation was not improved much even at 97% acetonitrile condition.

2) PC HILIC and ZIC HILIC in the second group are zwitterionic columns. The elution order looked similar and the signal intensity of smallest peak, MMP, was higher than those of the other columns including RP (Fig.1 c, d). ZIC HILIC, however, suffered from severe peak tailing. The possible reasons may be the differences in the direction of zwitterion on the surface of the resin. PC HILIC has phosphorylcholine and ZIC HILIC has sulfobetaine as a polar head group. The image of each zwitterion is shown in Figure 2. The surface of PC HILIC charged positive while that of ZIC HILIC negative. Because the phthalate monoesters have negative charge in common, they may tend to interact with positive charges of PC HILIC more strongly.

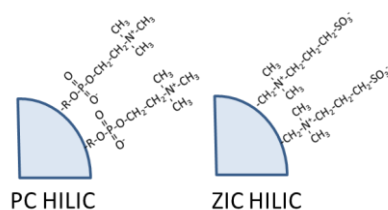


Figure 2. Functional group of PC and ZIC HILIC

3) XBridge HILIC and Atlantis HILIC silica in the third group have no modification on the silanol groups on the particle. The behavior of phthalate monoesters looked similar in both columns. The signal intensity and peak shape were both generally satisfactory (Fig.1 e, f). Due to absence of ionic interaction, their interactions with phthalate monoesters seem to be weaker than some of the other HILIC columns. In fact, concentration of organic solvent in the mobile phase had to be increased to 97% for obtaining good separation. Atlantis HILIC silica seems to have an advantage than other columns as for its potential to partially separate MEHP and MOP. Their separation on LC is essential because MOP shows signals in the same parent/daughter combination as MEHP.

4) Cosmosil HILIC and Shodex NH₂P in the last group have positive charge around neutral pH so that they may interact with the negatively-charged monoesters strongly. Therefore we tried diluted acetic acid or formic acid in aqueous mobile phase in order to attenuate the interaction. While virtually no improvements were observed by acetic acid in both columns, formic acid could improve the chromatogram of Cosmosil HILIC considerably (Fig.1g). As shown in figure, MEHP and MOP could be nearly separated. On the other hand, signal intensities were an order of magnitude lower than other column types. We tried to improve the sensitivity by changing buffers etc. but in vain. Shodex NH₂P was found to be even worse as for the signal intensities and peak shapes. Another drawback of Cosmosil HILIC is apparent spread of MEHHP peak. We do not know the reason of this spread.

In conclusion, some of the HILIC columns tested in the present research enabled sensitive analysis for phthalate monoesters due, possibly, to higher content of organic solvent in mobile phase. Among the HILIC columns tested, PC HILIC and Atlantis HILIC silica, followed by XBridge HILIC, were found to have benefits and/or potential for phthalate monoesters analysis by LCMSMS. Cosmosil HILIC may also have a potential,

although the reason of the low sensitivities in both of the cationic ion exchange columns are not clear at this stage. As unmodified bare silica gel tend to absorb variety of polar compounds non-specifically and might deteriorate faster when applying urine samples, we consider PC HILIC as a primary target of further research and will continue optimization of the whole clean-up and analysis condition together.

Acknowledgements

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References

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