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Optimization of Hydrophilic-Lipophilic Balance Solid-Phase Extraction of Phthalates in Pharmaceutical Preparations

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Abstract: Phthalates, which are used as plasticizers to soften rough polymers in the industrial processes, possess endocrine system disrupting activities. In this study, a hydrophilic-lipophilic balance solid-phase extraction method was optimized for seven phthalates as dimethyl, dipropyl, benzyl butyl, di-n-butyl, dicyclohexyl, di-(2-ethylhexyl) and di-n-octyl phthalates in terms of the type and the volume of the conditioning and the elution solvents. The phthalates were quantified by a validated HPLC/UV method. The recovery values were higher than 80% for dimethyl, dipropyl, benzylbutyl, di-n-butyl and dicyclohexyl phthalates. Using optimized conditions, three types of liquid pharmaceutical preparations as an intravenous isotonic sodium chloride solution, an intravenous dextrose solution and an osmotic laxative kept in polymeric packages were analyzed with high accuracy, precision and recovery. Only the intravenous isotonic sodium chloride solution was determined to be contaminated with dipropyl phthalate at a concentration of 13.2±0.16 µg/L.

Keywords: Hydrophilic-lipophilic balance, high performance liquid chromatography, pharmaceutical, phthalate, solid-phase extraction.

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INTRODUCTION

Phthalates are a class of dialkyl or alkyl aryl esters of 1,2-benzenedicarboxylic acid. They are suspected endocrine-disrupting compounds widely used to increase the flexibility of plastics or in common household products, cosmetics, detergents, flame retardants, plastics, inks, adhesives, metal food can liners, and medical devices (1). Since phthalates are not chemically bounded to polymer matrices, they can easily migrate to foods, beverages, cosmetics, and pharmaceuticals kept in polymeric packages (2).

Some of the phthalates are thought to disrupt the endocrine system by competing with 17β-estradiol for binding to the estrogen receptors (3). Also, toxicological studies have revealed that the lower molecular weight phthalates were irritating to eyes, nose, throat, and larger molecular weight phthalates were suspected carcinogens (1).

Considering the negative impacts of phthalates on human health, it became more of an issue to develop reliable extraction and analysis methods. Because of the low concentration levels and complex sample matrices, direct use of the analytical methods is usually limited by their sensitivity and/or selectivity. Generally, a pre-concentration/clean-up step is necessary prior to analysis. In this purpose, solid-phase extraction (SPE) is one of the mostly used methods in determination of phthalates (4). Different types of SPE sorbents such as PLRP-S (5), C18 (6), polymeric anion exchanger (7), florisil (8), carbon nanotubes (9), magnetic carbon nanotubes (10), magnetic graphene (11, 12) and molecularly imprinted polymers (13) have been used for the extraction of phthalates from various matrices. In a study, hydrophilic-lipophilic balance (HLB) sorbent, which is a hydrophilic modified polymeric reversed-phase material, was used to investigate the potential migration of plasticizers, plastic components and additives from several plastic water bottles (14). Since HLB is water-wettable, it possesses high retention capability even if the

sorbent runs dry. Also HLB contains both nonpolar and polar functional groups, that provides retention of a wide range of analytes from aqueous samples (4).

One of the reasons of the exposure to phthalates for human is the contaminated pharmaceutical preparations kept in polymeric packages. There are several studies on migration and/or determination of pharmaceutical packaging materials in the literature (15, 16). In the present study, it was aimed to determine the phthalate content of liquid pharmaceutical preparation samples in polymeric packages by an offline SPE-HPLC/UV method. A SPE procedure was optimized for the extraction of seven phthalates as dimethyl (DMP), dipropyl (DPP), benzyl butyl (BBP), di-n-butyl (DBP), dicyclohexyl (DCHP), di-(2-ethylhexyl) (DEHP) and di-n-octyl (DOP) phthalates using HLB cartridges. The analytes were detected by a simultaneous HPLC-UV method. Finally, the developed SPE-HPLC/UV method was applied to 3 different types of liquid pharmaceutical preparations as an intravenous isotonic NaCl solution, an intravenous dextrose solution, and an osmotic laxative preparation kept in polymeric packages.

MATERIALS AND METHODS

Materials and chemicals

The analytical standards of DMP, DPP, DBP, BBP, DCHP, DEHP, DOP and formic acid (FA) were purchased from Sigma (Darmstadt, Germany). The stock solution that contained each of the analyzed phthalates at a concentration of 10 mg/L was prepared with HPLC-grade methanol. The standard solutions in the range of 0.05–2.50 mg/L were prepared by diluting the stock solution to appropriate volumes with the mobile phase. Supel™-Select HLB SPE cartridges (200 mg/6 mL) were purchased from Sigma-Aldrich (Düren, Germany). Methanol (MeOH) (HPLC-grade), acetonitrile (ACN) (HPLC-grade), o-phosphoric acid (OPA) and potassium dihydrogen phosphate (KH_2PO_4) were purchased from Merck (Darmstadt, Germany). The phosphate buffer was prepared with 0.78 g KH_2PO_4 and 340 µL of OPA in 1 L of ultra pure water. All of the glassware used was rinsed with n-hexane and dried at 90°C to avoid any contamination of phthalates. Also a blank analysis was carried out to check the purity of the chemicals and SPE cartridge used.

Pharmaceutical preparation samples

Intravenous isotonic NaCl solution, intravenous dextrose solution, and osmotic laxative preparation in polymeric packages were purchased from a local drugstore in Istanbul (Turkey) in 2017.

Instruments and analytical conditions

The quantitative analysis of the phthalates was accomplished with a Shimadzu (Shimadzu, Kyoto, Japan) LC20A HPLC system with UV detection. Analytes were separated on a GL Sciences (GL Sciences, Tokyo, Japan) Intersil ODS-3 column (C18, 250×4.6 mm, i.d. 5.0 µm) and quantified by a slightly modified HPLC-UV method, which was developed and validated in a former study (2). A gradient program with a mobile phase system consisting of 0.2 M KH_2PO_4 buffer (pH 2.6) and MeOH/ACN (50:50, v/v) was established for the elution. The flow rate was 1 mL/min and the injection volume was set to 20 µL. The column temperature was adjusted to 40°C. The analyte peaks were detected at 230 nm. All of the analyses were performed in triplicate. The data obtained were analyzed by the LabSolutions software (version 1.25).

Quantification of phthalates

Phthalates were identified by comparing their retention times with those of the ones in the samples and with the increase of the peak areas after spiking. Data obtained using different wavelengths were compared. The quantification was performed by the external standard method. The calibration curves were prepared in the concentration range of 0.05–2.50 mg/L with six replicates. The linear regression model of least-squares was used for the calibration and analysis of the results (LabSolutions, Version 1.25).

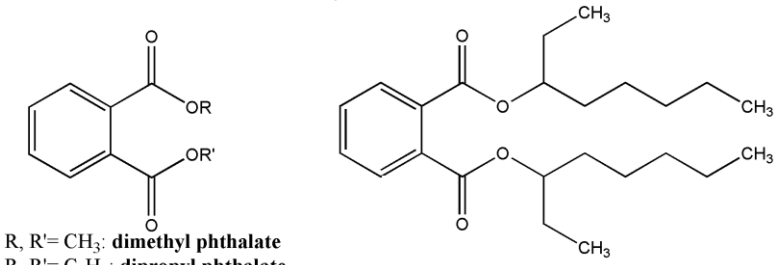
Optimization of the SPE method

At first, the SPE optimization studies were performed with a standard solution containing each of the phthalates at a concentration of 0.10 mg/L. The volume and the type of the conditioning and the elution solvents were optimized to obtain the highest recovery values. Several conditioning solvents (A: MeOH, water; B: MeOH; C: MeOH, 1% *o*-phosphoric acid solution; D: MeOH at pH 3.0 by *o*-phosphoric acid, water; E: MeOH at pH 3.6 by phosphate buffer) were examined to get the best retention. Three different types of elution solvents (MeOH/ACN, 50:50 v/v; ACN; ACN with 1% FA) were compared in terms of elution efficiency. The volumes of the conditioning and the elution solvents were selected following the trials performed with 3, 6 and 12 mL of each. Later, phthalates were extracted from the original and spiked (at 0.10 mg/L) liquid pharmaceutical preparation samples using the optimized SPE procedure. The standard solutions, original and spiked samples were extracted in triplicate to check the repeatability of the method. The precision of the method was determined as the percent relative standard deviation (RSD%) of the three replicate extractions. The recoveries from the standard solutions were calculated using the HPLC analysis data and the real concentration of the solutions. The recovery from the pharmaceutical preparation samples were determined by the data of the original and the spiked samples.

RESULTS AND DISCUSSION

In the present study, Supel™-Select HLB cartridges, which were composed of hydrophilic

modified styrene polymer, were used for the extraction of phthalates. One of the advantages of HLB sorbents for the extraction of analytes with aromatic rings is their selectivity to these compounds by π–π interactions. In addition, HLB sorbents possess both nonpolar and polar functional groups, providing the extraction of a wide range of polar to nonpolar and acidic to basic compounds from aqueous samples. Considering these advantages, an HLB SPE method was developed and applied for the extraction of DMP, DPP, BBP, DBP, DCHP, DEHP and DOP (Figure 1) from liquid pharmaceutical preparations kept in polymeric packages. The volume and type of the conditioning and the elution solvents were optimized to obtain the highest recovery values. Phthalates were quantified by a slightly modified HPLC-UV method, which was developed and validated by Yilmaz et al. (2). The analytical performance of the present HPLC-UV method was given in Table 1.



R, R'= CH_3: **dimethyl phthalate**
R, R'= C_3H_7: **dipropyl phthalate**
R, R'= C_4H_9: **di-n-butyl phthalate**
R= $C_6H_5CH_2$, R'= C_4H_9: **benzylbutyl phthalate**
R, R'= C_6H_{11}: **dicyclohexyl phthalate**
R, R' = C_8H_{17}: **di-n-octyl phthalate**

Figure 1. Chemical structures of the analyzed phthalates.

Selection of the conditioning solvent type

The general conditioning procedure for an HLB cartridge includes conditioning with an organic solvent like methanol (MeOH) or acetonitrile (ACN) followed by water or a buffer solution for aqueous samples. In the present study, several conditioning solvent types (A: MeOH, water; B: MeOH; C: MeOH, 1% *o*-phosphoric acid solution; D: MeOH at pH 3.0 by *o*-phosphoric acid, water; E: MeOH at pH 3.6 by phosphate buffer) were examined to get the best retention from a standard solution containing each of the phthalates at a concentration of 0.10 mg L^{-1} (Figure 2). Recovery values higher than 80.00% were obtained by using conditioning solvent E. Only DEHP and DOP had lower retention (recoveries 52.88% and 32.62%, respectively) since the polarities of these analytes were significantly lower than the others. After conditioning, 6 mL of the standard solution was loaded. The cartridge was washed with 3 mL of water to avoid the water soluble interferences. Then the analytes were eluted with 6 mL of ACN containing 1% FA.

Table 1. Analytical performance of the HPLC/UV method (*n* = 6).

Analyte	t_R (min)	Calibration range (mg/L)	Linear equation	R^2	LOD (mg/L)	LOQ (mg/L)	Tailing factor	Resolution
DMP	4.285±0.018	0.05-2.50	y=1016567x+7258.6	0.9999	0.01	0.02	1.167±0.035	-
DPP	6.589±0.035	0.05-2.50	y=504397x+4266.2	0.9999	0.01	0.02	1.145±0.049	12.018±0.162
BBP	7.589±0.095	0.05-2.50	y=632886x+4272.5	0.9999	0.01	0.02	1.137±0.085	5.704±0.055
DBP	7.897±0.033	0.05-2.50	y=290853x+3066.5	0.9999	0.01	0.03	1.141±0.063	2.057±0.063
DHCP	9.659±0.021	0.05-2.50	y=250847x+2352.1	0.9999	0.01	0.02	1.118±0.055	9.481±0.085
DEHP	13.581±0.045	0.05-2.50	y=2562203x+546.5	0.9999	0.01	0.02	1.102±0.065	17.764±0.046
DOP	14.415±0.019	0.05-2.50	y=241992x-2914.6	0.9977	0.09	0.27	1.110±0.078	2.941±0.084

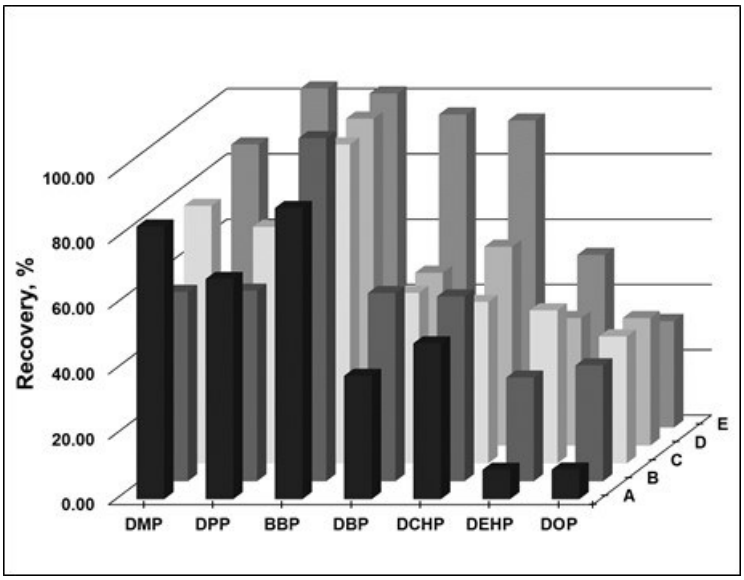


Figure 2. Selection of the conditioning solution. Loading: 6 mL of 0.10 mg/L standard solution, washing: 3 mL H_2O, elution: 6 mL ACN (1% FA). *n* = 3, RSD%: 0.62 – 2.35.

Selection of the elution solvent type

Three different types of elution solvents (MeOH/ACN, 50:50 v/v; ACN; ACN with 1% FA) were examined to optimize the SPE procedure. The highest recovery values were obtained with ACN containing 1% FA (Figure 3). Non-acidified elution solvents were incapable of breaking the interactions between the phthalates and the HLB sorbent sufficiently.

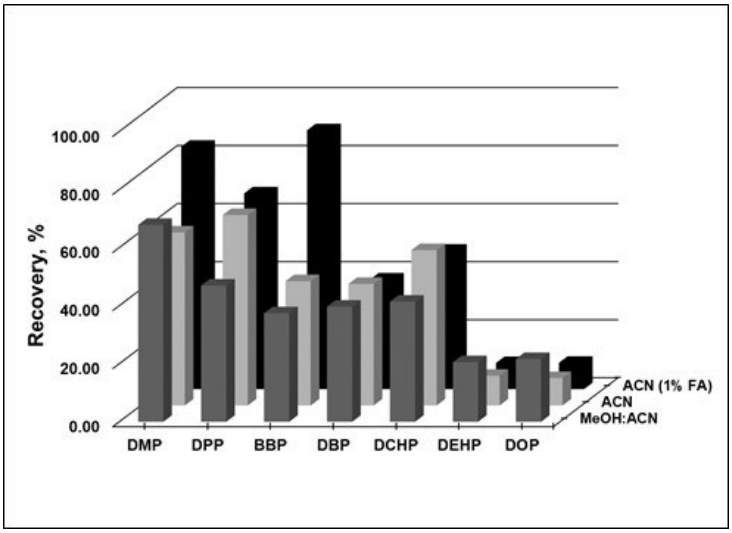


Figure 3. Selection of the elution solvent. Conditioning: 6 mL of MeOH **-** 6 mL of water, loading: 6 mL of 0.10 mg/L standard solution, washing: 3 mL H_2O. *n* = 3, RSD%: 0.74 – 2.14.

Selection of the conditioning and the elution solvents' volumes

Several studies were performed to optimize the volumes of conditioning and the elution solvents. For this purpose 3, 6, and 12 mL of conditioning and elution solvents were used for the extraction of the phthalates. Considering the recovery values, 6 mL of conditioning solvent (Figure 4) and 6 mL of elution solvent were selected as the optimum volumes (Figure 5). The volume values higher than 6 mL provided similar results of recovery.

Pharmaceutical preparation sample analysis

Using the optimized SPE procedure, phthalates were extracted from three different types of liquid pharmaceutical preparations as an intravenous isotonic NaCl solution, an intravenous dextrose solution, and an osmotic laxative preparation in polymeric packages. Six milliliters of the sample was loaded to HLB cartridge after conditioning with 6 mL of MeOH at pH 3.6 (adjusted by phosphate buffer). The sorbent was washed with 3 mL of water and the analytes were eluted using 6 mL of ACN containing 1% FA. The solvent was evaporated under reduced pressure and the residue was dissolved in 0.6 mL of the mobile phase. Twenty microliters of the filtered solution

(through 0.45 µm) was injected to the HPLC-UV system. The SPE procedure was triplicated for each of the original and the spiked (at 0.10 mg/L) samples. All of the HPLC-UV analyses were performed in triplicate. Precision of the method was determined in terms of relative standard deviation percent (RSD%), which was lower than 2.85% (Table 2). The recovery values of the phthalates were higher than 80% except DEHP and DOP (Table 2).

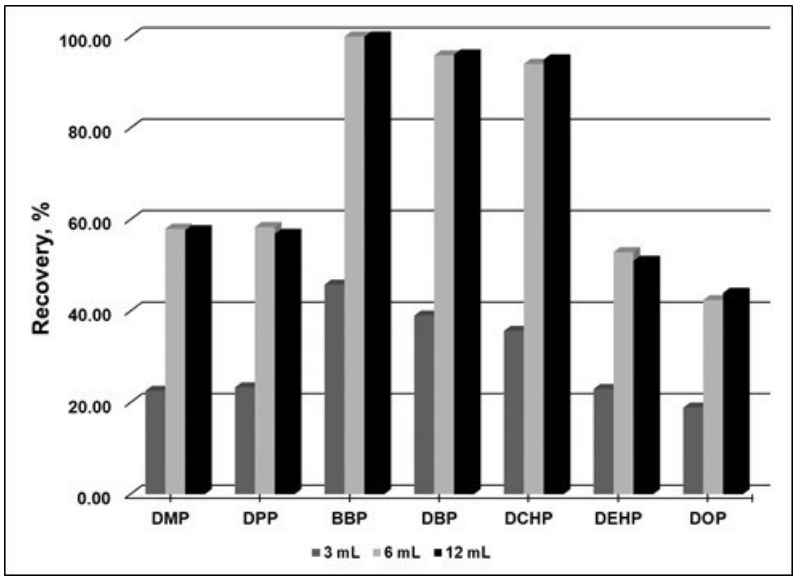


Figure 4. Selection of the conditioning solvent volume. Conditioning: 6 mL MeOH, loading: 6 mL 0.10 mg/L standard solution, washing: 3 mL H_2O, elution: 6 mL of ACN (1% FA). *n* = 3, RSD%: 0.52 – 2.11.

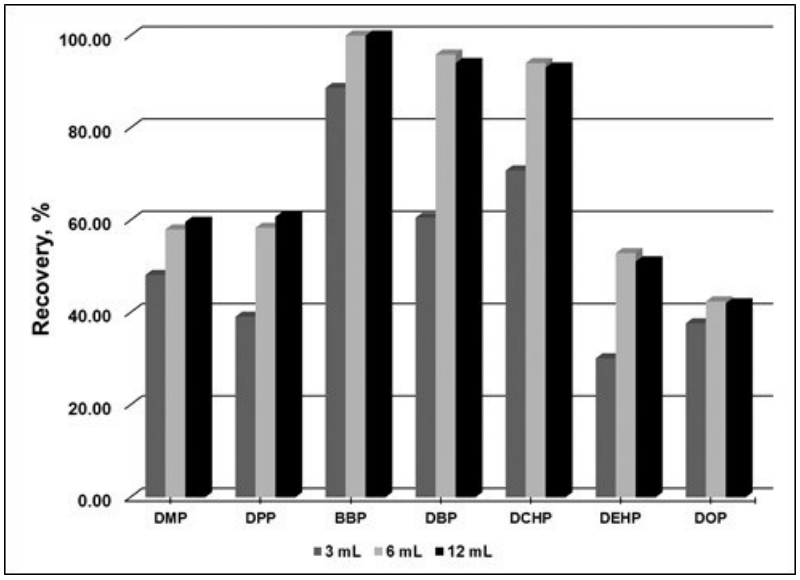


Figure 5. Selection of the elution solvent volume. Conditioning: 6 mL of MeOH, loading: 6 mL 0.10 mg/L std solution, washing: 3 mL of H_2O, elution: 6 mL ACN (1% FA). *n* = 3, RSD%: 0.96 – 2.41.

Table 2. Precision and accuracy of the SPE-HPLC/UV method*.

Analyte	Isotonic NaCl solution		Dextrose solution		Osmotic laxative	
	Recovery%	RSD%	Recovery%	RSD%	Recovery%	RSD%
DMP	92.45	2.36	103.48	1.99	108.96	1.68
DPP	96.33	2.59	100.65	1.86	90.21	2.85
BBP	87.21	1.89	96.55	2.10	90.25	2.01
DBP	86.66	2.45	88.23	1.98	86.63	1.68
DCHP	80.55	1.50	80.55	2.05	81.02	1.56
DEHP	50.88	1.02	54.72	2.01	53.12	2.01
DOP	45.77	2.05	40.52	2.54	41.22	2.11

* Samples were spiked at 0.10 mg/L (n = 3).

In other studies, DEHP and DBP were detected in pharmaceutical preparations kept in polyvinyl chloride and polyethylene packages, respectively (16, 17, 18, 19). Unlikely, the analyzed samples did not contain any of the phthalates examined in the present work, except isotonic NaCl solution which was determined as contaminated with DPP at 13.2±0.16 µg/L (RSD%, 1.24%). HPLC chromatograms belonging to original and spiked isotonic NaCl solution samples were given in Figure 6.

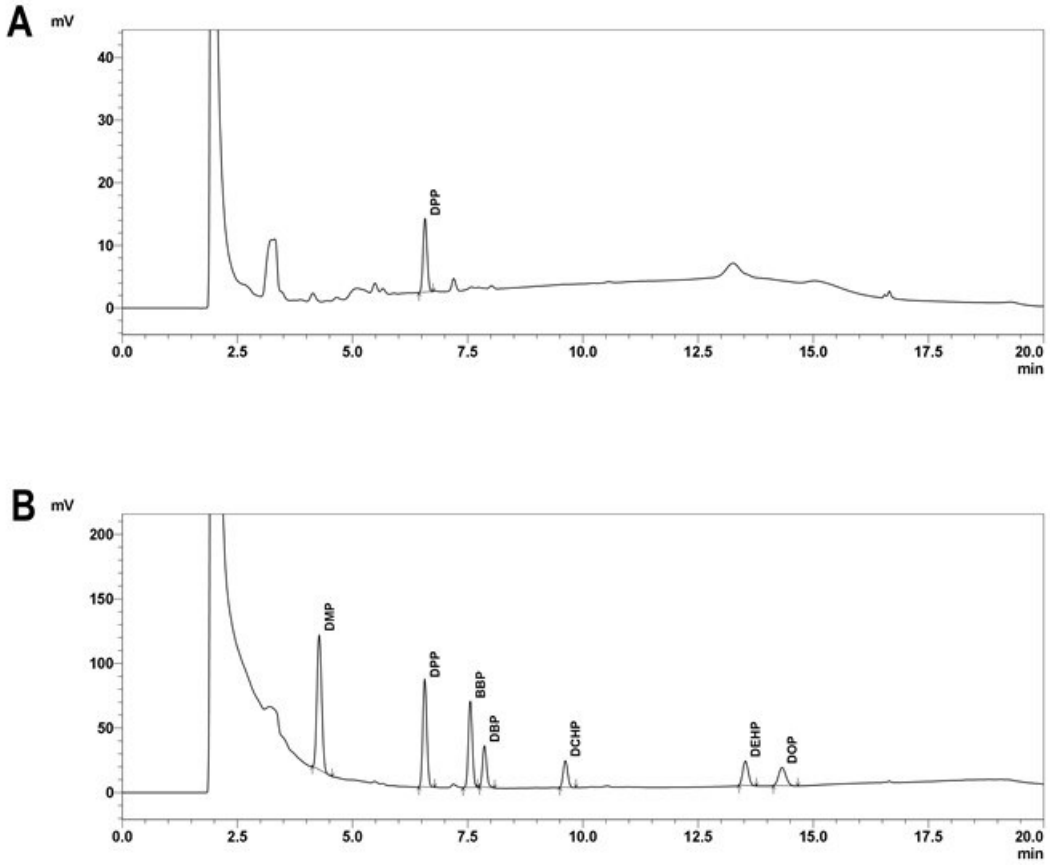


Figure 6. HPLC chromatograms of A) original and B) 0.10 mg/L spiked isotonic NaCl solution sample.

CONCLUSION

In the present study, an SPE method was optimized for the simultaneous extraction of seven phthalates from liquid pharmaceutical preparations by HLB cartridges. The optimized extraction method was capable of extraction of DMP, DPP, DBP, BBP and DCHP with recovery values higher than 80%. The extraction recoveries were below 80% for DEHP and DOP since they have lower polarities. A conditioning solvent with lower polarity might be more appropriate to improve the retention of DEHP and DOP by HLB sorbent. The optimized SPE method provided determination of low concentrations of phthalates (at level of µg/L) with good precision and accuracy using a common HPLC system with UV detection. To the best of our knowledge, the proposed work might be the first report on phthalate content of pharmaceuticals kept in polymeric packages in Turkey.

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