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Journal of Chromatography B, 789 (2003) 393–404
JOURNAL OF CHROMATOGRAPHY B
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Improved quantitative detection of 11 urinary phthalate metabolites in humans using liquid chromatography–atmospheric pressure chemical ionization tandem mass spectrometry

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Received 6 September 2002; received in revised form 3 February 2003; accepted 4 February 2003

Abstract

Phthalates are widely used as industrial solvents and plasticizers, with global use exceeding four million tons per year. We improved our previously developed high-performance liquid chromatography–atmospheric pressure chemical ionization-tandem mass spectrometric (HPLC–APCI-MS/MS) method to measure urinary phthalate metabolites by increasing the selectivity and the sensitivity by better resolving them from the solvent front, adding three more phthalate metabolites, monomethyl phthalate (mMP), mono-(2-ethyl-5-oxohexyl)phthalate (mEOHP) and mono-(2-ethyl-5-hydroxyhexyl)phthalate (mEHHP); increasing the sample throughput; and reducing the solvent usage. Furthermore, this improved method enabled us to analyze free un-conjugated mono-2-ethylhexyl phthalate (mEHP) by eliminating interferences derived from coelution of the glucuronide-bound, or conjugated form, of the mEHP on measurements of the free mEHP. This method for measuring phthalate metabolites in urine involves solid-phase extraction followed by reversed-phase HPLC–APCI-MS/MS using isotope dilution with $^{13}C_4$ internal standards. We further evaluated the ruggedness and the reliability of the method by comparing measurements made by multiple analysts at different extraction settings on multiple instruments. We observed mMP, monoethyl phthalate (mEP), mono-*n*-butyl phthalate (mBP), monobenzyl phthalate (mBzP), mEHP, mEHHP and mEOHP in the majority of urine specimens analyzed with DEHP-metabolites mEHHP and mEOHP present in significantly higher amounts than mEHP.

Keywords: Phthalates

1. Introduction

Many people are routinely exposed to phthalates (diesters of phthalic acid) because of their wide use as industrial solvents and plasticizers. After human exposure and absorption, phthalate diesters are metabolized to their respective monoesters and their oxidative products that are partially glucuronidated and excreted through urine and feces [1–5]. The metabolism is reported to be rapid, with a large portion being excreted within a short time [2,6]. The proportion of the diester that is converted in vivo to

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1570-0232/03/$ – see front matter
doi:10.1016/S1570-0232(03)00164-8

its specific monoester or other oxidative metabolite is phthalate-dependent [6]. Exposure to dibutyl phthalate (DBP) and diethyl phthalate (DEP) resulted in excretion of their respective monoesters as the primary metabolites, whereas for di(2-ethylhexyl) phthalate (DEHP), the oxidative metabolites predominate. Some phthalates and their metabolic products are responsible for reproductive [7,8] and developmental toxicities in animals [9,10]. However, little information is known about the effects of phthalate exposure on humans. To understand any adverse health outcomes associated with phthalate exposure, reliable information about the exposures must be obtained. In exposure assessment of suspected toxic chemicals, measurement of internal dose produces valuable information [11,12]. Hence, urine and serum are widely used as matrices for measuring the internal dose of toxic chemicals. Both phthalate diesters [4,13,14] and their respective monoesters (Fig. 1) [1,15,16] have been used as urinary or serum biomarkers of phthalate exposure. We recently published the urinary levels of metabolites of selected phthalates in non-representative [1,15] and representative [17] US populations. Measurable levels of the monoesters of DEP and DBP [1,15,17], which are widely used in many consumer products such as perfumes, cologne, soap, shampoo, nail polish and cosmetics, were reported. Low levels of monoesters of more hydrophobic diesters such as dicyclohexyl phthalate (DCHP), DEHP, DiNP, and DOP were also reported [1,17], indicating either low exposure, bioaccumulation or different path of metabolism or excretion compared with more hydrophilic diesters.

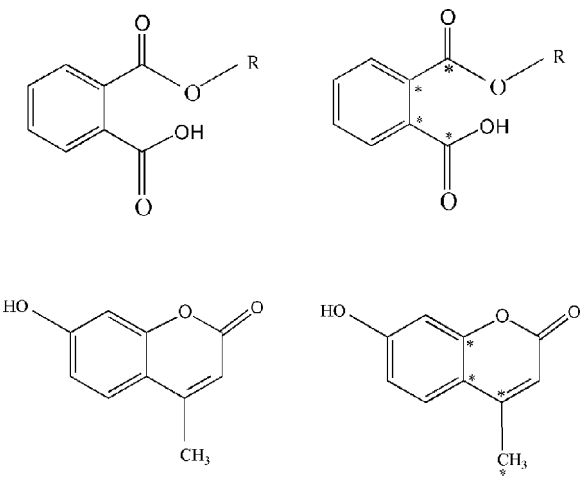

Fig. 1. (*A) The generalized chemical structures of phthalate monoesters, (B) their internal standards, (C) 4-methyl-umbelliferone and (D) its internal standard. *Indicates the position of labeled ^{13}C.

We previously developed a sensitive high-performance liquid chromatography–atmospheric pressure chemical ionization-tandem mass spectrometric (HPLC–APCI-MS/MS) method to assess exposure to phthalates using monoesters as the biomarkers for exposure [18]. We modified this method to include three important additional analytes, to greatly improve the chromatography of low-molecular mass hydrophilic analytes, to better resolve them from the solvent front, to analyze free un-conjugated mEHP by eliminating the interferences derived from coelution of the glucuronide-bound form (or conjugated form) of the mEHP on measurements of the free mEHP, to increase the sample throughput of the method and to make it cost effective. We expanded the method to measure 11 phthalate metabolites, monomethyl phthalate (mMP), monoethyl (mEP), mono-*n*-butyl (mBP), monocyclohexyl (mCHP), monobenzyl (mBzP), mEHP, mono-*n*-octyl (mOP), mono-3-methyl-5-dimethylhexyl (*iso*-nonyl, mNP), and mono-3-methyl-7-methyloctyl phthalate (*iso*-decyl, mDP), mono-(2-ethyl-5-oxohexyl)phthalate (mEOHP) and mono-(2-ethyl-5-hydroxyhexyl)phthalate (mEHHP) in human urine with the detection limits in the low ng/ml (Table 1) range using $^{13}C_4$-labeled analytes as the internal standards (Fig. 1) for nine of the above analytes while $^{13}C_4$ mBP is using as the internal standard for DEHP-metabolites mEOHP and mEHHP. In the analysis of total phthalate monoesters, the completion of the deglucuronidation was monitored as a quality assurance step by monitoring the deglucuronidation of 4-methyl-umbelliferryl-glucuronide.

2. Experimental

2.1. Reagents

Analytes mMP, mEP, mBP, mCHP, mBzP, mEHP, mOP, mNP, mDP, mEOHP and mEHHP (>99.9%), $^{13}C_4$-stable isotope-labeled internal standards of

Table 1
The phthalate metabolites, their parent and daughter masses set for Q1 and Q3 scans, collision energies (CE), their respective retention times (RT) from Waters 2690 and HP 1100 HPLC systems, SPE recoveries and the detection limits (DL)

Monomethyl phthalate (mMP)	R[a]	Parent mass	Daughter mass	CE(V)	RT, min Waters 2690	RT, min HP1100	SPE Rec. (%)	DL (ng/ml)
Monomethyl phthalate (mMP)	$-CH_3$	179	107	17.5	3.14	3.10	62.4	0.70
Monoethyl phthalate (mEP)	$-C_2H_5$	193	121	16.0	3.76	4.63	76.6	1.2
Monobutyl phthalate (mBP)	$-C_4H_9$	221	77	22.0	5.67	6.49	91.5	0.94
Monocyclohexyl phthalate (mCHP)	$-C_6H_{11}$	247	77	24.5	6.56	7.32	93.3	0.93
Monobenzyl phthalate (mBzP)	$-CH_2C_6H_5$	255	183	14.2	6.74	7.44	91.3	0.47
Mono-2-ethylhexyl phthalate (mEHP)	$-CH_2CH(C_2H_5)CH_2(CH_2)_2CH_3$	277	134	19.0	10.4	10.5	90.6	0.86
Mono-(2-ethyl-5-oxohexyl) phthalate (mEOHP)	$-CH_2CH(C_2H_5)CH_2CHCOCH_3$	293	121	20.0	5.59	6.40	88.5	1.2
Mono (2-ethyl-5-hydroxyhexyl) phthalate (mEHHP)	$-CH_2CH(C_2H_5)CH_2CH(OH)CH_3$	291	121	20.0	5.85	6.55	88.5	1.6
Mono-*n*-octyl phthalate (mOP)	$-CH_2(CH_2)_6CH_3$	277	125	19.7	10.95	9.75	87.5	0.77
Monoisononyl phthalate (mNP)	$-CH_2CH_2CH(CH_3)CH_2C(CH_3)_3$	291	247	16.5	11.0	9.84	89.0	0.79
Monoisodecyl phthalate (mDP)	$CH_2CH_2CH(CH_3)CH_2(CH_2)_2CH(CH_3)_2$	305	155	16.4	11.85	10.6	77.2	0.50

[a] Ref. Fig. 1A.

mMP, mEP, mBP, mCHP, mBzP, mEHP, mOP, mNP, mDP (>99.9%, Fig. 1) and $^{13}C_4$ 4-methyl-umbelliferone internal standard (Fig. 1) were purchased from Cambridge Isotope Laboratories (Andover, MA, USA). Acetonitrile and water (HPLC grade), phosphoric acid (85%), ethyl acetate (99.8%), monosodium phosphate monohydrate (ultrapure bioreagent), ammonium hydroxide (30%), 4-methylumbelliferone (Fig. 1), its glucuronide and ammonium acetate (>98%) were purchased from Tedia (Fairfield, OH, USA), Fisher Scientific (Pittsburgh, PA, USA), Caledon (Ontario, Canada), J.T. Baker (Phillipsburg, NJ, USA) and Sigma (St. Louis, MO, USA), respectively. β-Glucuronidase (*Escherichia coli*-K12) was purchased from Roche Biomedical (Mannheim, Germany). APCI sheath gas (liquid nitrogen dewar head space) and collision gas (argon, ultrapure carrier grade) were purchased from Holox (Atlanta, GA, USA). Reagents were prepared in acetonitrile and water using standard laboratory procedures. All standard solutions were prepared in glassware that was methanol-rinsed and dried.

2.2. Standard preparation

Stock solutions of native standards (phthalate monoester metabolites and 4-methylumbelliferone) and internal standards ($^{13}C_4$-labeled phthalate monoesters) were prepared and stored as previously described [18]. A stock solution of $^{13}C_4$-labeled 4-methylumbelliferone was prepared in water. Eleven unique standard solutions of phthalate monoesters and 4-methylumbelliferone were prepared in water from the stock solutions of native and internal standard (approx. 1–2500 ppb). Stock standard solutions were stored at −20 °C in PTFE-lined bottles. The working standards were stored at 4 °C. The calibration curves were prepared directly from pure standards and internal standards in water.

2.3. Sample preparation

Human urine (1.00 ml) was measured into a borosilicate glass test tube (16×125 mm, Corning) and buffered with ammonium acetate (250 μl, 1 *M*, pH 6.5). The urine was spiked with a mixture of labeled phthalate internal standards (12–50 ng), 4-methylumbelliferyl glucuronide [19] and 4-methylumbelliferone internal standard (50 ng). When measuring total phthalate concentrations, β-glucuronidase enzyme (5 μl) and ammonium acetate buffer [18] (250 μl) were added to each sample to deconjugate glucuronidated phthalate metabolites. The samples were sealed with PTFE-lined screw caps and gently mixed and incubated at 37 °C for 90 min for total phthalate analysis. To measure only the

Table 2
HPLC gradient program used to separate of mMP, mEP, mBP, mCHP, mBzP, mEHP, mEOHP, mEHHP, mOP, mNP and mDP

Time (min)	0	1.0	10.0	11.0	11.2	11.5	12.0
%A[a]	100	85	55	0	0	100	100
%B[b]	0	15	45	100	100	100	0

The flow-rate was set to 0.8 ml/min.
[a] One ml acetic acid in 1 l HPLC-grade water.
[b] One ml acetic acid in 1 l acetonitrile.

free phthalate concentrations, the enzyme deconjugation step was omitted, and samples were extracted immediately after adding the ammonium acetate buffer.

2.4. Solid-phase extraction

The solid-phase extraction (SPE) procedure was changed from the previous set-up [18]. Following the sample preparation step, the samples were processed through 60 and 200 mg Nexus SPE cartridges (Varian Sample Preparation Products, Harbor City, CA, USA) using solvents and buffered aqueous solutions. Two to three serially connected vacuum manifolds equipped with single-use PTFE flow lines (Supelco, Bellefonte, PA, USA) or Gilson Model 215 automated SPE system (Gilson, Middleton, WI, USA) were used for extractions.

The spiked urine samples were treated with ammonium hydroxide basic buffer (1 ml, 30% NH_4OH solution in 200 ml of 50:50 acetonitrile–water) and passed through preconditioned 60 mg Nexus SPE cartridges to remove hydrophobic compounds. The urine was collected, acidified by adding 3.0 ml phosphate buffer, pH 2.0 (0.14 *M* NaH_2PO_4 in 0.85% H_3PO_4), and vortex mixed. The SPE cartridges were discarded. A preconditioned 200 mg Nexus SPE cartridge was used to retain the analytes. The acidified urine was passed through the SPE cartridge, and the eluate was discarded. The analytes were then eluted from the cartridge with acetonitrile (2 ml) followed by ethyl acetate (2 ml). The combined eluates were concentrated under a stream of dry nitrogen (UHP grade) in a Turbovap evaporator (Zymark, Hopkinton, MA, USA) at 55 °C. The residue was resuspended in 200 μl water and transferred to autosampler vials. The samples were then analyzed by HPLC–APCI-MS/MS.

2.5. Instrumental analysis

The chromatographic separation was achieved using a Waters Alliance 2690 HPLC (Milford, MA, USA) or an Agilent 1100 HPLC (Wilmington, DE, USA), each equipped with a Betasil phenyl column (5 μm, 50 mm×2 mm, Keystone, Bellefonte, PA, USA) with a nonlinear solvent gradient from 100% mobile phase A (0.1% acetic acid in water) to 100% mobile phase B (0.1% acetic acid in acetonitrile) at 0.6 ml/min (Table 2). The mass specific detection was achieved using a ThermoFinnigan TSQ 7000 (San Jose, CA, USA) equipped with an APCI interface or a PE Sciex API 3000 (Applied Biosystems, Foster City, CA, USA) equipped with heated nebulizer interface. The TSQ 7000 was controlled by Xcalibur software and the API 3000 was controlled by Analyst software. Each sample (25 μl) was injected using the HPLC autosampler, configured with syringe washes between injections. Inline filters (2 and 0.5 μm, Upchurch Scientific, Oak Harbor, WA, USA) were used to filter particulates from the injected samples before reaching the column. Both mobile phases were prepared fresh every other day. APCI in negative ion mode was used to form negatively charged analyte ions at the interface. The mass spectrometers were tuned and optimized for each analyte at 0.6 ml/min flow-rate. The following settings of the TSQ 7000 were used for analysis: nitrogen sheath gas (40 p.s.i.), API vaporizer temperature (500 °C), heated capillary temperature (250 °C), corona needle discharge (9 μA), tube lens voltage (182 V), Q_0 (7 V), electron multiplier (1800 V), and collision-induced dissociation (CID) gas pressure (2.0 mTorr). Data acquisition and analysis on the TSQ 7000 were performed using Xcalibur software on a PC-based data system. The following settings of the API 3000 were used for analysis: nitrogen curtain gas setting (9), corona

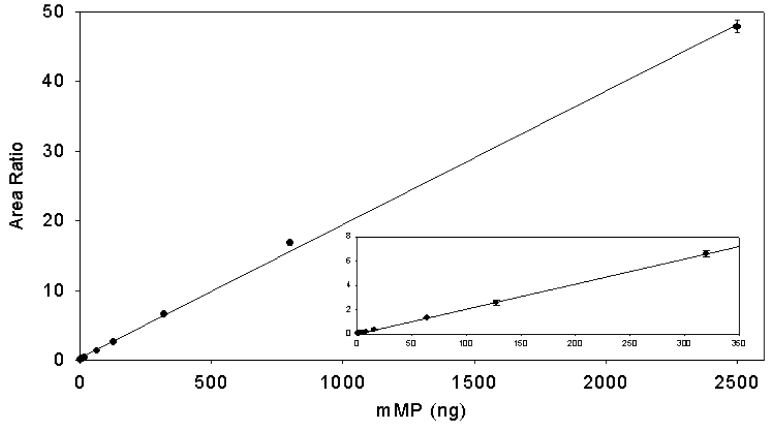

Fig. 2. A sample daily calibration curve for mMP representing all analytes. The calibration curve was linear across the range from 0 to 2500 ppb with correlation coefficient typically exceeding 0.99.

needle voltage setting (−5) Nebulizer gas setting (14), collision gas setting (2). Data acquisition and analysis on the API 3000 were controlled by the Analyst software on a PC-based data system.

The instruments were set in daughter ion mode, and the parent and daughter ion combinations specific to the eluting analyte were monitored (Table 1). The identity of the monoesters was confirmed by matching retention times (±2%) with the $^{13}C_4$-labeled internal standard (Fig. 1). The quantification was done using the isotope dilution method [19]. The identity of the mEHHP and mEOHP was confirmed by matching the retention time of the standard to the unknown. Data analyses were performed as previously described [18].

2.6. Daily operation and quality control procedure

Quality control (QC) materials were prepared from pooled urine collected from multiple anonymous donors. The pooled urine was mixed well and then split equally into two smaller pools. The pools were spiked at high (QCH, 30–500 ng/ml) and low (QCL, 15–100 ng/ml) levels with the phthalate metabolites. Each pool was characterized by a minimum of 100 repeat determinations over a 2-month period to determine the mean and 95th and 99th confidence limits for both free and total phthalate concentrations (i.e., with and without an enzyme hydrolysis). QC materials were analyzed during each analytical run to ensure proper operation of the

Table 3
Mean concentrations and the %C.V. (coefficient of variation) of repeat manual or automated extractions of QCH pools containing mMP, mEP, mBP, mCHP, mBzP, mEHP, mOP and mNP by three different analysts

Analyte	Analyst A SPE			Analyst B SPE			Analyst C SPE		
	n	Conc. (ppb)	%C.V.	*n*	Conc. (ppb)	%C.V.	*n*	Conc. (ppb)	%C.V.
mMP	107	213.5	7	67	211.6	7	48	213.0	9
mEP	113	478.3	6	61	477.7	5	51	480.8	6
mBP	122	105.2	8	67	105.9	7	52	106.4	8
mCHP	106	106.1	5	69	106.1	5	50	106.2	5
mBzP	102	130.4	10	67	127.9	9	45	129.3	10
mEHP	125	33.4	12	57	33.1	9	46	34.2	12
mOP	108	210.8	14	64	212.8	12	49	212.7	11
mNP	108	255.3	10	64	259.8	7	49	261.9	10

method and the validation of the resulting data. QC data were evaluated using Westgard QC rules [20].

Each analytical run consisted of one reagent blank, 1 QCH, 1 QCL, and 21 unknown samples. All samples were extracted simultaneously on the vacuum manifold or the automated extractor. After analysis, QCs and unknown samples were corrected for the reagent blank, and a QC check was performed. All 21 unknowns in the manifold were re-extracted if the QC failed for a particular analyte. If an individual sample failed the 4-methylumbelliferone QC check, only that unknown sample was re-extracted.

The calibration curve was derived daily from two full sets of 11 standards analyzed at the beginning and the end of the run sequence. Each point in the calibration curve was weighted ($1/x$), with correlation coefficients exceeding 0.99 (Fig. 2). A Microsoft Excel file of final data was exported to a Microsoft Access database, and the data were statistically analyzed using SAS statistical software (SAS Institute, Cary, NC).

2.7. Limits of detection

The standard deviation of repeated extractions of the five lowest standards were plotted against their concentrations. The analytical limit of detection (LOD) for each of the 11 analytes (Table 1) was calculated as $3S_0$, where S_0 is the value of the standard deviation as the concentration approaches zero. The intercept of the best-fit line of this plot was used to estimate S_0 [21].

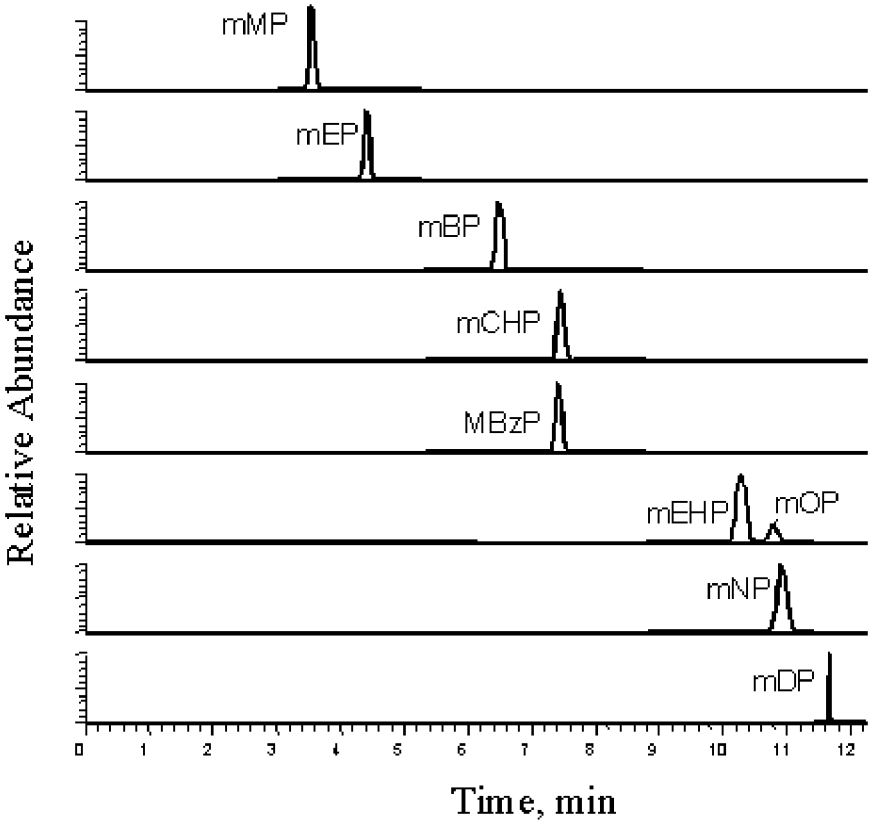

Fig. 3. Sample HPLC–APCI/MS/MS chromatogram for a standard mixture of mMP, mEP, mBP, mCHP, mBzP, mOP, mEHP, mNP and mDP using Keystone Betasil phenyl column (5 μm, 50×2 mm) with a nonlinear solvent gradient from 100% Buffer A (0.1% acetic acid in water) to 100% Buffer B (0.1% acetic acid in acetonitrile) at 0.6 ml/min flow-rate.

2.8. Recoveries

The recoveries were calculated by using the ratio of the amount of analytes recovered after SPE to the amounts originally added. The internal standard mixture containing $^{13}C_4$-labeled analytes were added after the drying step to account for any instrumental variation among the injected samples and to calculate the recovered amount (Table 1).

2.9. Method validation

2.9.1. Cross-instrument and cross-method comparisons

A set of known standards and QCs were repeatedly analyzed using both instrumental configurations, and both analytical methods and the data were compared.

2.9.2. SPE cross analyst comparison

Over 45 QCL and QCH pools were extracted manually using SPE vacuum manifolds by three different analysts for cross analyst comparison (Table 3).

2.9.3. SPE: manual versus automated

Ten QCL and QCH control pools were extracted manually using vacuum manifold and automatically using an automated solid-phase extractor (Gilson) for SPE comparison.

3. Results and discussion

We modified our method for measuring urinary phthalate metabolites (Fig. 1) in humans to greatly improve the overall performance of the method. We

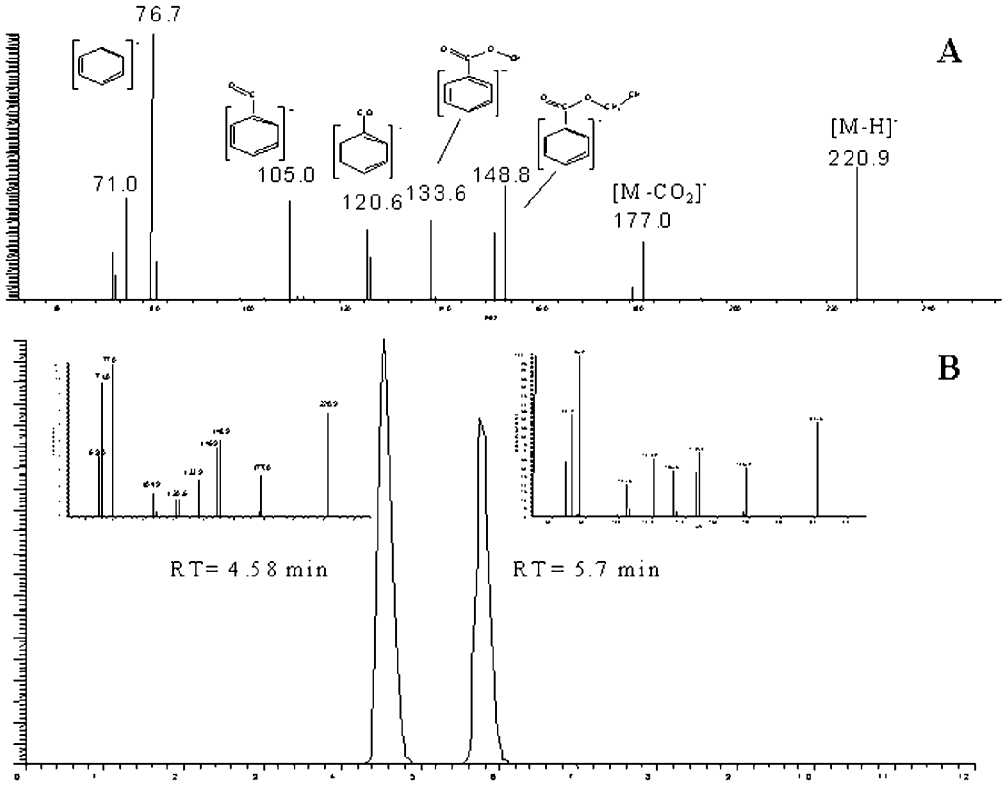

Fig. 4. (A) The mass spectrum of mBP. (B) The chromatogram of a human urine sample extracted without enzyme treatment for free phthalate analysis showing two peaks with MS/MS corresponding to 221/77 with identical mass spectra to mBP at RT=4.58 min and 5.7 min representing the elution of mBP-Glu and mBP, respectively.

obtained linear calibration curves for all analytes over three orders of magnitude with correlation coefficient exceeding 0.99 (Fig. 2). The use of an appropriate chromatographic condition prior to mass spectrometric analysis was necessary in order to determine all phthalate monoester analytes in one chromatographic run. Our previous method [18] with acetate buffer mobile phase at pH 6.7 and linear solvent gradient, produced poor separation of low-molecular mass phthalate monoesters. Therefore a non-linear gradient system from aqueous to organic mobile phase was developed to separate all tested phthalates as can be seen in Table 2. Furthermore, in our modified method, we used a lower flow-rate and lower mobile phase pH, which resulted in longer retention of free monoesters on the column, essentially eliminating the early eluting interferences that we previously observed. Under these conditions, the phenyl column retained more hydrophilic phthalate monoesters such as mMP and mEP, which originally eluted near or at the solvent front producing interferences from co-eluting species. This modification to the method, enabled us to include relatively hydrophilic mMP to our assay and to completely eliminate the interferences on mEP analysis. A typical chromatogram of a standard mixture with nine analytes is shown in Fig. 3.

Furthermore, our method has been used to quantify free monoesters by omitting the enzyme step in the extraction. The SPE effectively extracts both glucuronide-bound and the free form of the monoesters. However, upon ionization in the APCI source, the corona discharge facilitates partial dissociation of the conjugated monoester to form the free species

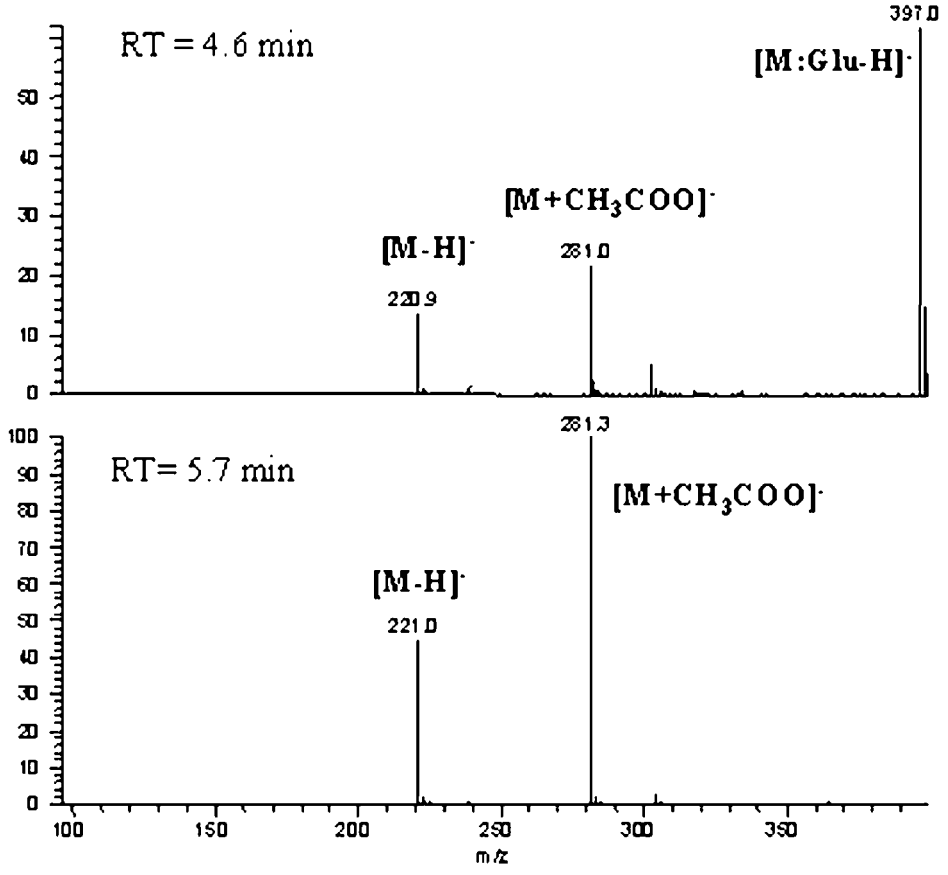

Fig. 5. Parent scan for $[M-1]^- = 221$(mBP) peaks at RT=4.6 and 5.7 indicating the mBP-glucuronide ($[M:Glu-H]^-$, 397) as the major contributor for mBP at RT=4.7, whereas at RT=5.7, the major contributor for mBP was the acetate adduct ($[M+CH_3COO]^-$, 281).

(i.e., in-source fragmentation, Fig. 4A,B). Consequently, both the glucuronide-bound and free monoesters produced identical full scan mass spectra similar to the free monoester metabolite (Fig. 5). The chromatographic separation of the glucuronide-bound monoester from the free form is therefore important to accurately measure the free monoesters in the matrix. Lowering the pH of the mobile phase closer to or below the pK_a values of the analytes suppressed the ionization, improving the chromatographic separation of the analytes and their respective glucuronides, allowing more accurate quantification of free monoesters, especially mEHP, in the absence of enzyme treatment (Fig. 6). With our cleaner separation of the glucuronide-bound and free monoesters, we observed a shift in their elution order (Fig. 6A,B). The new elution order (i.e., glucuronide-bound monoester eluting first) appeared reasonable because the glucuronide should be more water soluble.

Although we were able to improve the chromatographic retention and separation of monoesters and glucuronides by suppressing ionization, it resulted in fewer charged species in the ion source, producing poorer signal. To achieve the desired ion density at the MS interface, we charged the corona needle with a higher current.

The total run time for this method was increased by about 4 min per sample. However, with the previous method, about 25% of the samples had to be re-injected because of interferences with mEP, and often the volume available for repeat injections was low. So ultimately, the slight increase in retention time essentially reduced the number of samples we had to reanalyze. For studies with large sample numbers (e.g., more than 1000 samples), this improved method is more time efficient, cost effective, and environmentally friendly. The decreased flow-rate saves solvents and improves the life of the vacuum pumps used with the mass spectrometers. In addition, the SPE sample throughput was increased by 33% from the previous method by switching to a different sorbent.

For analytes, mMP, mEHHP and mEOHP, the full

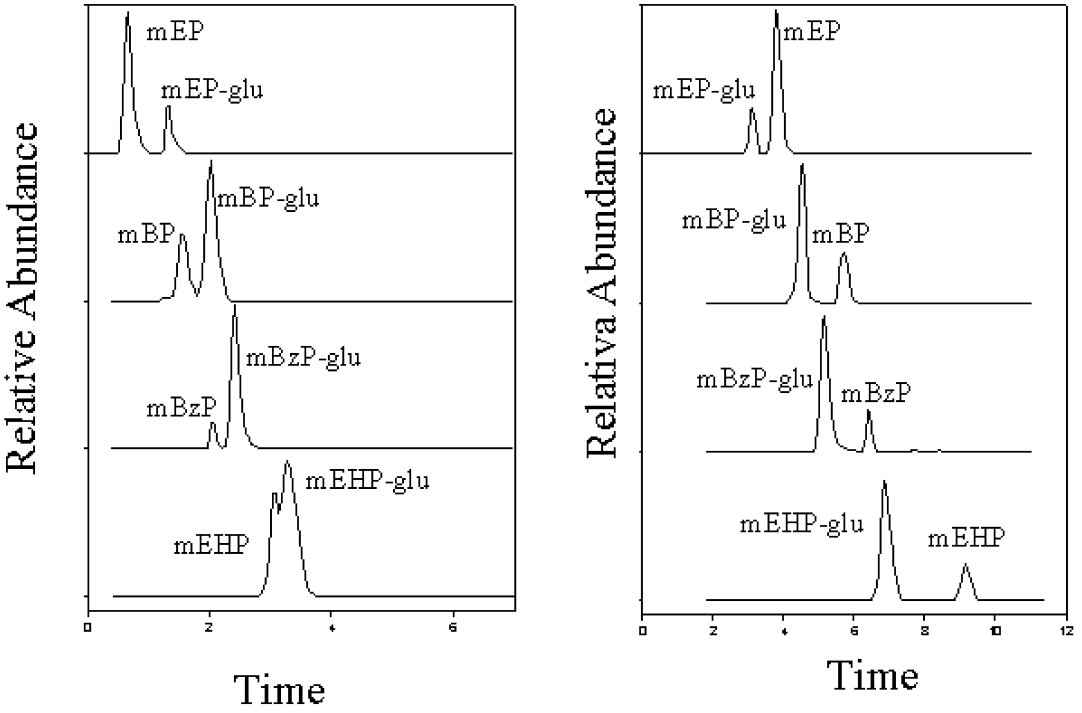

Fig. 6. Sample chromatograms of human urine extracts showing the elution pattern of mEP, mBP, mBzP, mEHP, and their glucuronides when processed without enzyme treatment. (A) Using previous method, (B) using modified method.

scan spectra of the parent compound were obtained to decide best parent daughter combinations. The mass spectrometer parameters were optimized for each parent/daughter combination to produce maximum levels of specific daughter ions. mBP produced similar recoveries and close retention time to the two DEHP metabolites making the ^{13}C-labeled mBP a good candidate for the internal standard for the quantification of mEHHP and mEOHP (Table 1). For the monoester metabolites, the recoveries were similar to the previous method and were uniform throughout the whole spectrum of standards. The slopes of the standard curves were virtually unchanged for all metabolites regardless of whether we analyzed the standards directly or after matrix-based extractions. This proves both the analytes and internal standards extract uniformly during SPE keeping the analyte to internal standard ratio constant. This enabled us to derive the calibration plots from fresh standards for quantification of unknown samples.

In our comparison studies, the data for analytes common to both methods agreed closely (Fig. 7A). In addition, agreement was excellent between multiple instruments (Fig. 7B,C), multiple analysts (Table 3) and multiple SPE methods was excellent (Fig. 7D) proving our method to be robust and rugged.

This improved method for quantifying urinary phthalate monoesters has been used in several epidemiological studies and in the National Health and Nutrition Examination Survey 2000 (NHANES-IV 2000) for assessing US population levels of phthalate monoesters [17]. We detected measurable levels of

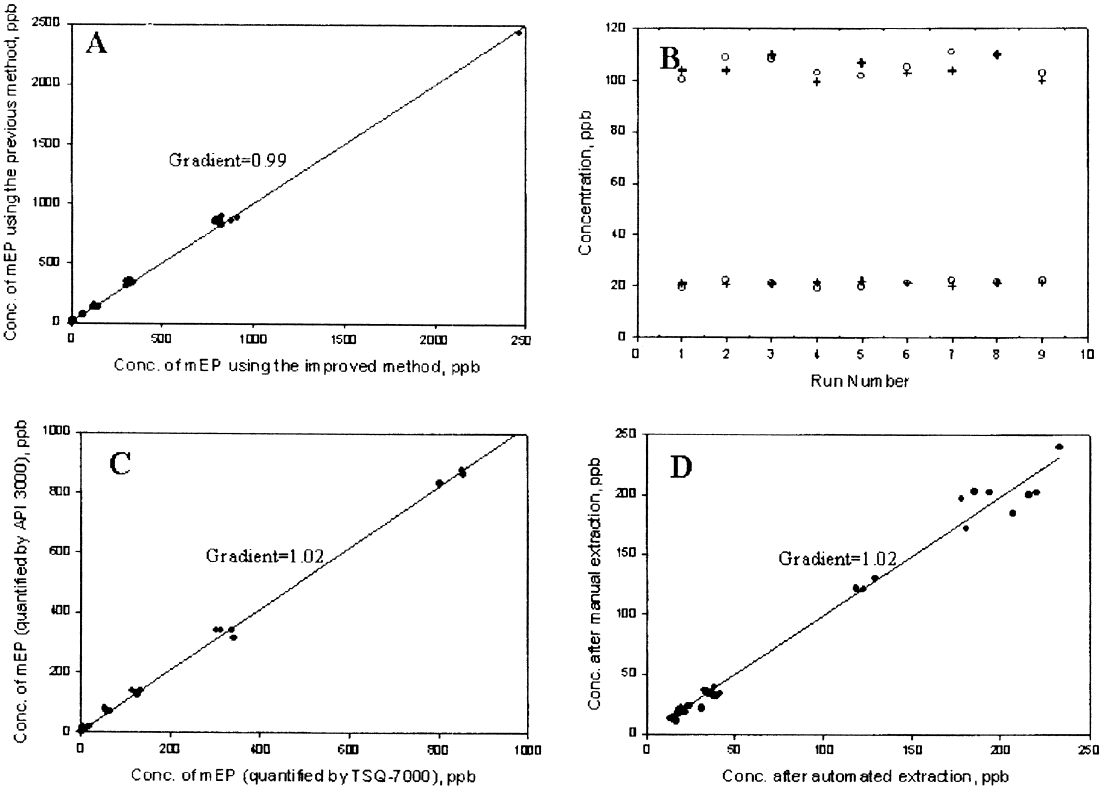

Fig. 7. (A) Repeat analyses of a series of mEP standards using our previous method and the current improved method. The gradient of 0.99 indicates an excellent match between two methods. (B) Concentrations of mBP from repeat extractions of QC High and QC Low analyzed by TSQ 7000 (+) and SciEx API 3000 (○). (C) Repeat analyses of a series of mEP standards using TSQ-7000 and SciEx API 3000. The gradient of 1.02 indicates a very close match between the two instruments. (D) Concentrations of different analytes from repeat extractions of QCH and QCL using manual versus automated extraction methods. The gradient of 1.02 indicates a close match between the two extraction methods.

diester phthalate metabolites, namely mMP in 80%, mEP in 100%, mBP in 98%, mEHP in 81%, and mBzP in 97.5% of the NHANES 2000 samples we analyzed. However, we infrequently detected mNP and mOP (<5%). These data demonstrate that our method is a useful tool for assessing exposure to the parent diester phthalates of mMP, mEP, mBP, mEHP, mBzP, mNP, mOP, mEHHP and mEOHP (Fig. 8). mEHHP, mEOHP and mDP were not analyzed in the NHANES-IV 2000 samples. However, in other epidemiologic studies we observed the two oxidative DEHP metabolites, mEHHP and mEOHP at significantly higher levels than DEHP metabolite mEHP [22].

When an authentic standard can be obtained, we anticipate applying this method to quantify the glucuronide-bound monoesters without significant modifications, to study detoxification and excretion of phthalates through glucuronidation pathway.

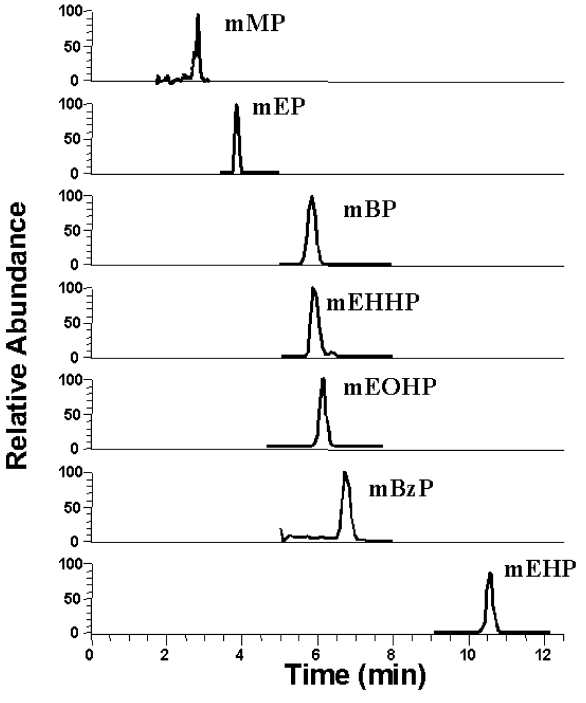

Fig. 8. Sample HPLC–APCI/MS/MS chromatogram from a human urine sample showing mMP, mEP, mBP, mEHHP, mEOHP, mBzP and mEHP.

Acknowledgements

The authors acknowledge Antonia Calafat for her input in this project. The use of trade names is for identification only and does not constitute endorsement by the US Department of Health and Human Services or the Centers for Disease Control and Prevention.

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