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Molecularly imprinted solid phase extraction of urinary diethyl thiophosphate and diethyl dithiophosphate and their analysis by gas chromatography–mass spectrometry

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ABSTRACT

An analytical method involving molecularly imprinted solid phase extraction (MISPE) and gas chromatography–mass spectrometry (GC–MS) was developed for the analysis of organophosphates metabolites (diethyl thiophosphate – DETP and diethyl dithiophosphate – DEDTP) in human urine samples. A DETP molecularly imprinted polymer (MIP) was synthesized using 4-vinylpiridine as the functional monomer and ethylene glycol dimethacrylate as the cross-linker. The conditioning step of the MISPE was conducted by running 3 mL of acetonitrile, 3 mL of 0.1 mol L^{-1} dibasic phosphate buffer at pH 11 and 2 mL of water through the molecularly imprinted polymer (MIP) cartridge. The extraction step was executed using 1.0 mL of a urine sample, with the pH previously adjusted to 3.0. Finally, the analytes were eluted with 3 mL of acetonitrile and derivatized with 3% 2,3,4,5,6-pentafluorobenzyl bromide solution at room temperature for 1 h. The sample was analyzed by GC–MS in the SIM (selected ion monitoring) mode. Analytical calibration curves for DETP and DEDTP were constructed using a pool of urine samples and six levels of concentration. The method was found to be linear from 10 to 500 μg L^{-1} ($r > 0.99$) with limits of quantification of 10 μg L^{-1} for both analytes. The within-day and between-day precisions were evaluated (as %RSD) and all the results were <15% for both analytes. The method was accurate (relative error < ±15%), with good robustness.

1. Introduction

Organophosphate pesticides (OPs) have been widely and effectively used as insecticides, with many applications in agricultural and residential settings [1]. In humans, these compounds are metabolized to dialkyl phosphates (DAPs) and excreted in the urine (80–90% of the total dose within 48 h) [2,3]. Urinary DAPs, such as dimethyl phosphate (DMP), diethyl phosphate (DEP), dimethyl thiophosphate (DMTP), diethyl thiophosphate (DETP), dimethyl dithiophosphate (DMDTP) and diethyl dithiophosphate (DEDTP), are commonly used as biomarkers of organophosphate exposure [4,5]. Fig. 1 shows the chemical structure of DETP and DEDTP, the main urinary metabolites of the dissulfoton.

The determination of DAPs by liquid chromatography has not been a common strategy due the difficulty in separating these metabolites by this technique [5]. Several methods based on the gas chromatographic determination of the derivatized DAP metabolites can be easily found in the literature [3–5]. However, due to the presence of several concomitants in the urine samples, the use of efficient extraction techniques is necessary to avoid interference problems during the derivatization and chromatographic analyses. In this way, some specific techniques can be pointed out, such as liquid–liquid extraction [6–9], solid phase extraction [10,11], ionic exchange extraction [12], extractive derivatization [13], azeotropic distillation [14–16] and lyophilization [17–20], among others. However, when unspecific methods are used, some concomitants may remain [14]. An efficient alternative for circumventing this problem is the use of selective sorbents such as molecularly imprinted polymers (MIPs) [21–23]. MIPs are cross-linked synthetic polymers obtained by the copolymerization of a functional monomer with a cross-linker in the presence of a template molecule. After polymerization, the polymer is washed. Removal of the template leaves specific recognition sites in the polymer that are complementary to the template in terms of size, shape and chemical functionality. The imprinted polymer is able to selectively rebind the template molecule (analyte) and

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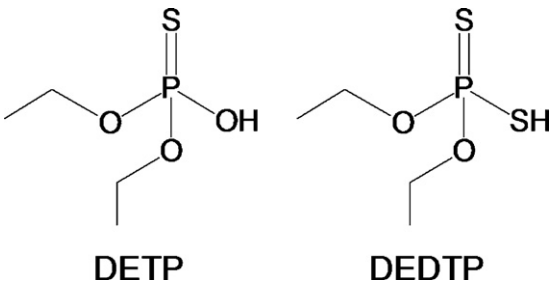

Fig. 1. Dialkyl phosphate metabolites.

other molecules with analogous structures [24,25]. MIPs have been extensively used in the solid phase extraction of several analytes in different matrices [26–30].

Based on the relevant characteristics of MIP compared to other adsorbents, such as selectivity, sensitivity, high stability, high lifetime, and low cost, we perceived, for the first time, the possibility of synthesizing a MIP for selective extraction of DETP and DEDTP in urine samples, followed by the separation and quantification of these analytes using gas chromatography–mass spectrometry (GC–MS).

2. Experimental

2.1. Chemicals and solutions

All the HPLC grade (99.99%) organic solvents, such as acetonitrile, tetrahydrofuran, hexane and methanol were obtained from Vetec (Rio de Janeiro, Brazil). The solutions were prepared with deionized water (18.2 MΩ cm) from a Milli-Q water purification system (Millipore, Bedford, USA). For the MIP synthesis, DETP, 4-vinylpyridine, ethylene glycol dimethacrylate (EGDMA), 2,2′-azobisisobutyronitrile (AIBN) (all from Sigma–Aldrich, Steinheim, Germany) were used as template, functional monomer, crosslinking reagent and initiator, respectively. Acetonitrile was used as the solvent. A solution of methanol:acetic acid (Merck, Darmstand, Germany) at a ratio of 9:1 (v/v) was used during the washing of the MIP to remove the template. Stock solutions of DETP and DEDTP (Sigma–Aldrich, Steinheim, Germany) were prepared at 1.0 mg L^{-1} in HPLC grade acetonitrile, placed in an amber flask and kept at −20 °C for up to 30 days. Working solutions of 0.1–500 μg L^{-1} were prepared daily by diluting the standard solution in acetonitrile. A solution of 2,3,4,5,6-pentafluorobenzyl bromide (PFBBr) (Sigma–Aldrich, Steinheim, Germany) in acetonitrile was used as the derivatization reagent.

2.2. Gas chromatography and mass spectrometry conditions

The electron ionization (70 eV) mass spectrometric analysis was performed using a GC–MS QP-2010 from the Shimadzu® Corporation (Kyoto, Japan) equipped with a RTx®-5MS (30 m × 0.25 mm i.d. × 0.25 μm) capillary column (RESTEC, Bellefonte, USA). Pure helium (99.999%) with a column flow of 1.53 mL min^{-1} was used as the carrier gas. A 2 μL aliquot of the standard/sample was injected in the splitless mode and analyzed under the following conditions. The initial temperature of the column was maintained at 140 °C for 1 min, raised to 230 °C at 40 °C min^{-1} and maintained at 230 °C for 1 min. The column temperature was then raised to 260 °C at 20 °C min^{-1}, raised to 300 °C at 40 °C $min^{-1}$and maintained at 300 °C for 0.5 min. The injector, interface and ion source temperatures were 270, 300 and 230 °C, respectively. The quantitative analysis was achieved in selected ion monitoring mode (SIM) with an event time of 0.2 s for each analyte. The ions at *m*/*z* 350 and *m*/*z* 366 were used to quantify DETP and DEDTP, respectively. The ions at *m*/*z* 350, 274 and 213 as well as the ions at *m*/*z* 366, 185 and 157 were employed to confirm the identity of DETP and DEDTP, respectively. The data files were acquired with the GCMS-Solution software®.

2.3. MIP synthesis

The synthesis of the DETP-imprinted polymer was based on non-covalent interactions between the template and the functional monomer. In a 25 mL glass flask, 1 mmol of DETP and 4 mmol of 4-vinylpyridine were dissolved in 6 mL of acetonitrile and this solution was sonicated at room temperature. After 5 min, 16 mmol of EGDMA and 0.75 mmol of AIBN were added, and the mixture was purged with nitrogen in the ultrasonic bath for 10 min. The flask was sealed and immersed into a water bath at 65 °C for 24 h. After polymerization, the monolith obtained was mechanically ground, and the particle size was selected using a steel sieve (75–106 μm). The particles were transferred to a glass flask and washed in an ultrasonic bath with 4:1 (v/v) methanol/acetic acid for 1.5 h. The washing procedure was repeated 10 times, and the washing solution was renewed for each repetition. The efficacy of template removal was checked analyzing the eluates of the washing solutions by GC–MS until nothing could be detected. The polymer particles were dried at 70 °C, and 50 mg were packed in polypropylene cartridges. The non-imprinted polymer (NIP) was synthesized in the same way as the MIP, in the absence of the template.

2.4. Sample preparation and MISPE procedure

The urine sample handling was approved by the Ethics Committee of the Federal University of Alfenas (no. 296/2010).

Initially, 3 mL of acetonitrile, 3 mL of 0.1 mol L^{-1} dibasic phosphate buffer at pH 11.0 and 2 mL of water were flowed through the MIP cartridge at 1 mL min^{-1} during the conditioning step. Then, 1.0 mL of human urine sample at pH 3.0 (adjustment with 0.1 mol L^{-1} nitric acid aqueous solution) was percolated through the cartridge at 1 mL min^{-1}, and DETP and DEDTP were selectively extracted. Finally, the analytes were eluted with 3 mL of acetonitrile, and the extract was evaporated to dryness under a nitrogen stream. The derivatization reaction was performed according to the procedure described by De Alwis et al. [5], with some modifications. K_2CO_3 (15.0 mg) and 250 μL of 3% PFBBr solution (v/v in acetonitrile) were added to the tube containing the residue from the MISPE. The extract was allowed to stand for 1 h at room temperature for the derivatization reaction. Then, the derivatized extract was evaporated to dryness under a nitrogen stream and reconstituted in 100 μL of tetrahydrofuran before the GC–MS analysis.

2.5. Validation study

The following validation parameters were evaluated: linearity, sensitivity, precision, accuracy, recovery, detection and quantification limits and robustness. This study was performed using a pool of blank human urine samples spiked with DETP and DEDTP. The linearity and sensitivity were established through the calibration curve obtained by a sextuplicate analysis of DETP and DEDTP at six concentration levels (10, 50, 100, 200, 350 and 500 μg L^{-1} for DETP and DEDTP). Linearity and sensitivity were expressed as the correlation coefficient (*r*) and the slope of the calibration curve, respectively. Intra-assay precision and accuracy were assessed with five replicates from each concentration level (10, 50, 200 and 500 μg L^{-1} for DETP and DEDTP) on the same day. Inter-assay precision and accuracy were evaluated by three replicates analyzed at each concentration level (10, 50, 200 and 500 μg L^{-1} for both analytes) on separate days. The results were expressed as percent relative standard deviations and percent relative errors for

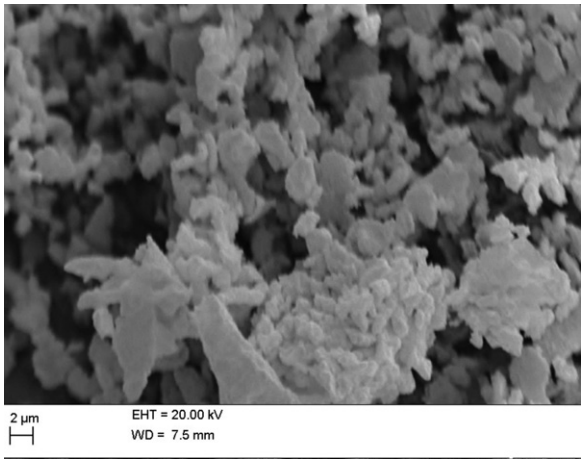

Fig. 2. Scanning electron micrographs of the MIP.

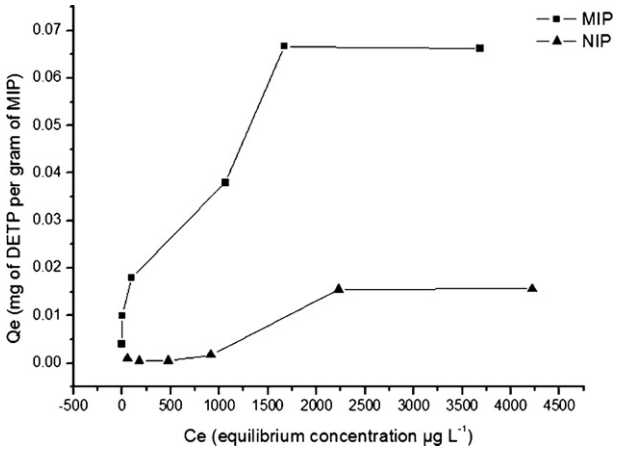

Fig. 3. Adsorption isotherms of DETP for the MIP and the NIP.

precision and accuracy, respectively. The recovery was calculated using the extraction efficiency by comparing the responses obtained after the analysis of a fortified blank urine sample ($n = 3$) and a fortified extract obtained after the MISPE procedure (with a blank urine sample). The limits of detection (LODs) and quantification (LOQs) were calculated based on the standard deviation (SD) and slope (S) of the calibration curve and in accordance with the following formulas: LOD = 3.3 (SD/S) and LOQ = 10 (SD/S). Ruggedness was evaluated using the Youden approach, which is based on a fractional factorial design.

3. Results and discussion

3.1. MIP characterization

The synthesis provided approximately 3 g of material. The morphological structure of the MIP can be observed in Fig. 2, which shows the scanning electron microscopy (SEM) image of the material. The MIP showed high porosity. This characteristic is important in extraction procedures because it favors interactions between the analytes and the absorbent [31].

The extraction capabilities of the MIP and NIP were also investigated. DETP aqueous solutions, ranging in concentration from 0.1 to 7.0 µg L^{-1}, were extracted as described in Section 2.4 and the mass of the DETP retained in the polymer was calculated. Fig. 3 shows the adsorption isotherm for DETP in both the polymers. It indicates that the adsorption for both the materials increased when the DETP concentration increased. As observed, the MIP had the highest adsorption capacity, probably because its interactions with DETP were based on molecular recognition, whereas only non-specific interactions prevailed between the NIP and DETP [31,32].

Table 1
Validation parameters of the MISPE GC–MS method for the determination of DETP and DEDTP in urine.

Validation parameters	DETP	DEDTP
Linear range (µg L^{-1})	10–500	10–500
Correlation coefficient (r)	0.9978	0.9925
Slope (L µg^{-1})	591.96	1014.95
LOD (µg L^{-1})	3.0	3.0
LOQ (µg L^{-1})	10.0	10.0
Recovery (extraction efficiency) (%)	28.9[a]	32.7[a]
	29.4[b]	31.9[b]
	29.7[c]	32.3[c]

[a] For 50 µg L^{-1} ($n = 5$).
[b] For 200 µg L^{-1} ($n = 5$).
[c] For 500 µg L^{-1} ($n = 5$).

In addition, a linear Langmuir model was employed to describe the adsorption behavior of DETP in the MIP. A good fit was observed ($r = 0.97$) as shown in Fig. 4, and the adsorptive maximum capacity was 67 µg g^{-1} (calculated as the inverse of the slope). The Langmuir model explains a uniform distribution of the binding sites around the polymer and the analytes are retained in a monolayer on the MIP surface [33]. After saturation, the DETP has more affinity for the solvent than for other molecules already adsorbed [34].

3.2. Optimization of the MISPE conditions

The following variables were evaluated in a univariate manner: urine pH, mass of the polymer and nature/volumes of the solvents for the conditioning and elution steps. The starting conditions were as follows: a cartridge with 50 mg of MIP, conditioning with 3 mL of acetonitrile, loading with 1 mL of urine at physiological pH and elution with 1 mL of acetonitrile.

Initially, the pH of the standards/samples was investigated from 1 to 9 and by using 0.1 mol L^{-1} nitric acid and 0.1 mol L^{-1} sodium hydroxide aqueous solutions to adjust it. Different behaviors were observed for both analytes and the best results, in terms of absolute analytical signal, were in acidic (pH smaller than 3.0) and basic (pH larger than 6.0) medium for DETP and DEDTP, respectively. In this way, the pH was optimized at 3.0 as a compromise between the sensitivities for both analytes. Probably the electrostatic interactions prevail between the analytes and the functional monomer at this pH, once the DETP, DEDTP and 4-vinylpyridine are in their ionized forms.

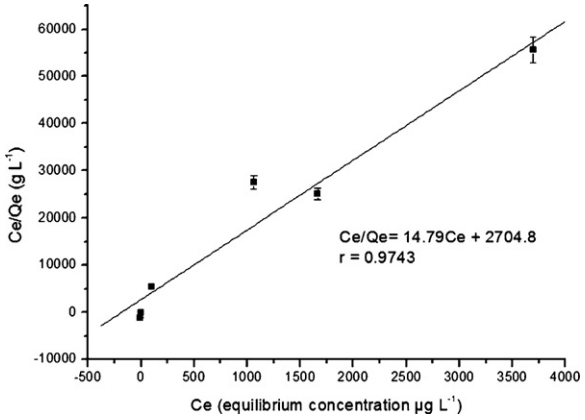

Fig. 4. Langmuir adsorption isotherm of DETP for the MIP.

Table 2
Precision and accuracy for the MISPE GC–MS analysis of DETP and DEDTP in urine samples.

		DETP				DEDTP			
Within-day ($n=5$)	NC[a] ($\mu g\,L^{-1}$)	10[e]	50	200	500	10[e]	50	200	500
	AC[b] ($\mu g\,L^{-1}$)	11.1	50.4	217.9	505.8	8.9	53.5	171.2	496.2
	Precision (RSD[c], %)	11.3	13.5	7.6	11.2	19.4	11.0	14.2	12.7
	Accuracy (E[d], %)	10.7	0.8	9.0	7.1	−11.3	2.2	−14.4	−0.8
Between-day ($n=4.3$)	NC ($\mu g\,L^{-1}$)	10[e]	50	200	500	10[e]	50	200	500
	AC ($\mu g\,L^{-1}$)	10.9	47.2	194.7	501.4	8.3	51.1	170.2	503.4
	Precision (RSD, %)	15.5	9.3	12.2	6.9	19.2	5.3	3.8	3.2
	Accuracy (E, %)	8.9	−5.6	−2.6	0.3	−17.5	2.2	−14.9	0.7

[a] Nominal concentration.
[b] Analyzed concentration.
[c] Coefficient of variation.
[d] Relative error.
[e] LOQ.

The washing step after loading was not necessary, ever since the presence of interferences was not observed. Certainly, the MIP was able to retain exclusively the template instead of other concomitants from urine, evidencing the effectiveness of the selective binding sites.

The mass of the MIP in the cartridge was evaluated in terms of increase in the analytical signal. When the mass of the MIP was increased from 25 to 50 mg, no significant changes were observed in the analytical signal for DETP. On the other hand, when the mass was increased from 50 to 150 mg, the analytical signals of DETP increased 25%. For DEDTP, a decrease in the analytical signal (ca. 86%) was observed when the mass of MIP was increased from 50 to 150 mg. This fact probably was a consequence of the insufficient volume of the elution solvent, resulting in an incomplete elution of this analyte from these larger masses of the polymer. When 25 mg was employed, there was a decrease of the DEDTP response, probably due to the insufficient material for the retention. The best result for DEDTP was obtained for 50 mg and this mass was selected as working condition.

The choice of elution solvent is very important to guarantee the efficient elution of the analytes from the MIP. For DETP, an increase of 20% in the analytical signal was observed when methanol was used as eluent in comparison with acetonitrile. For DETDP, the analytical signal decreased 56% when the elution was done with methanol in comparison with acetonitrile. Because acetonitrile is slightly more lipophilic than methanol, it was more efficient for eluting DEDTP (more lipophilic due to the presence of an SH group [35]). Along these lines, the best responses were obtained with methanol for DETP and acetonitrile for DEDTP, and the acetonitrile was optimized for further experiments as a compromise between the sensitivities of both analytes. Ethyl acetate and tetrahydrofurane were also investigated, but they were not able to elute the analytes from MIP. The volume of acetonitrile was studied from 0.5 to 3.0 mL and increases in the analytical signal of 45% for DETP and of 10% for DEDTP were observed when 3 mL of acetonitrile was used, possibly because larger volumes were more efficiently distributed in the MIP cartridge, improving the elution. Then, 3 mL was selected for further experiments.

Conditioning solutions are necessary to prepare the adsorbent for the next extraction, mainly by the elimination of analytes and concomitants coming from previous extractions. Firstly, 3 mL of acetonitrile was used as the conditioning solution. However, the complete removal of the analytes (from the previous extraction) was not guaranteed with this solution, especially for DEDTP, resulting in a memory effect. As observed during the sample pH optimization, the interactions between the MIP and DEDTP were weaker in a basic medium. Thus, different alkaline solutions of 0.1 mol L^{-1} of dibasic phosphate buffer at a pH ranging from 6.0 to 11.0 was passed through the MIP (3 mL) as a second conditioning step (after acetonitrile). Almost complete cleaning was obtained with 0.1 mol L^{-1} dibasic phosphate buffer at pH 11.0, and this solution was then selected. Finally, 2 mL of water was flowed through the MIP (after phosphate buffer solution), and the analytes and other concomitants were completely eliminated from the polymer, avoiding the memory effect.

Fig. 5 shows the chromatograms obtained after MISPE optimization for 500 $\mu g\,L^{-1}$ of DETP and DEDTP aqueous standard, a blank urine sample and a urine sample fortified with 500 $\mu g\,L^{-1}$ of DETP and DEDTP. As observed, the selectivity of MISPE was evidenced due to the absence of concomitant peaks when the blank urine sample was analyzed by SIM. Fig. 6A and B shows the mass spectra for DETP and DEDTP, respectively.

3.3. Confidence parameters and method application

Table 1 shows the linear range, correlation coefficient, slope, LOD, LOQ and recovery for the DETP and DEDTP analysis by MISPE and GC–MS. As observed, the results attest to the good performance of the present method for the DETP and DEDTP analysis in human urine samples. About the low values of recoveries, it is important to highlight that this fact was not a limiting factor, since the method showed adequate precision and accuracy (see Table 2), and

Table 3
Analytical parameters and its variations evaluated by Youden test.

Analytical parameters	Nominal (+)	Variation (−)	Factorial combination for assay								Formula to variation effect
			1	2	3	4	5	6	7	8	
Injector temperature (°C)	270.0	255.0	+	+	+	+	–	–	–	–	$(a+b+c+d)/4-(e+f+g+h)/4$
Source temperature (°C)	230.0	219.0	+	+	–	–	+	+	–	–	$(a+b+e+f)/4-(c+d+g+h)/4$
Interface temperature (°C)	300.0	285.0	+	–	+	–	+	–	+	–	$(a+c+e+g)/4-(b+d+f+h)/4$
Total flow rate (mL min^{-1})	20.0	19.0	+	+	–	–	–	–	+	+	$(a+b+g+h)/4-(c+d+e+f)/4$
Sample pH	3.0	2.85	+	–	+	–	–	+	–	+	$(a+c+f+h)/4-(b+d+e+g)/4$
Elution volume (mL)	3.0	2.8	+	–	–	+	+	–	–	+	$(a+d+e+h)/4-(b+c+f+g)/4$
Derivatization reagent volume (mL)	0.25	0.24	+	–	–	+	–	+	+	–	$(a+d+f+g)/4-(b+c+e+h)/4$
Result			a	b	c	d	e	f	g	h	

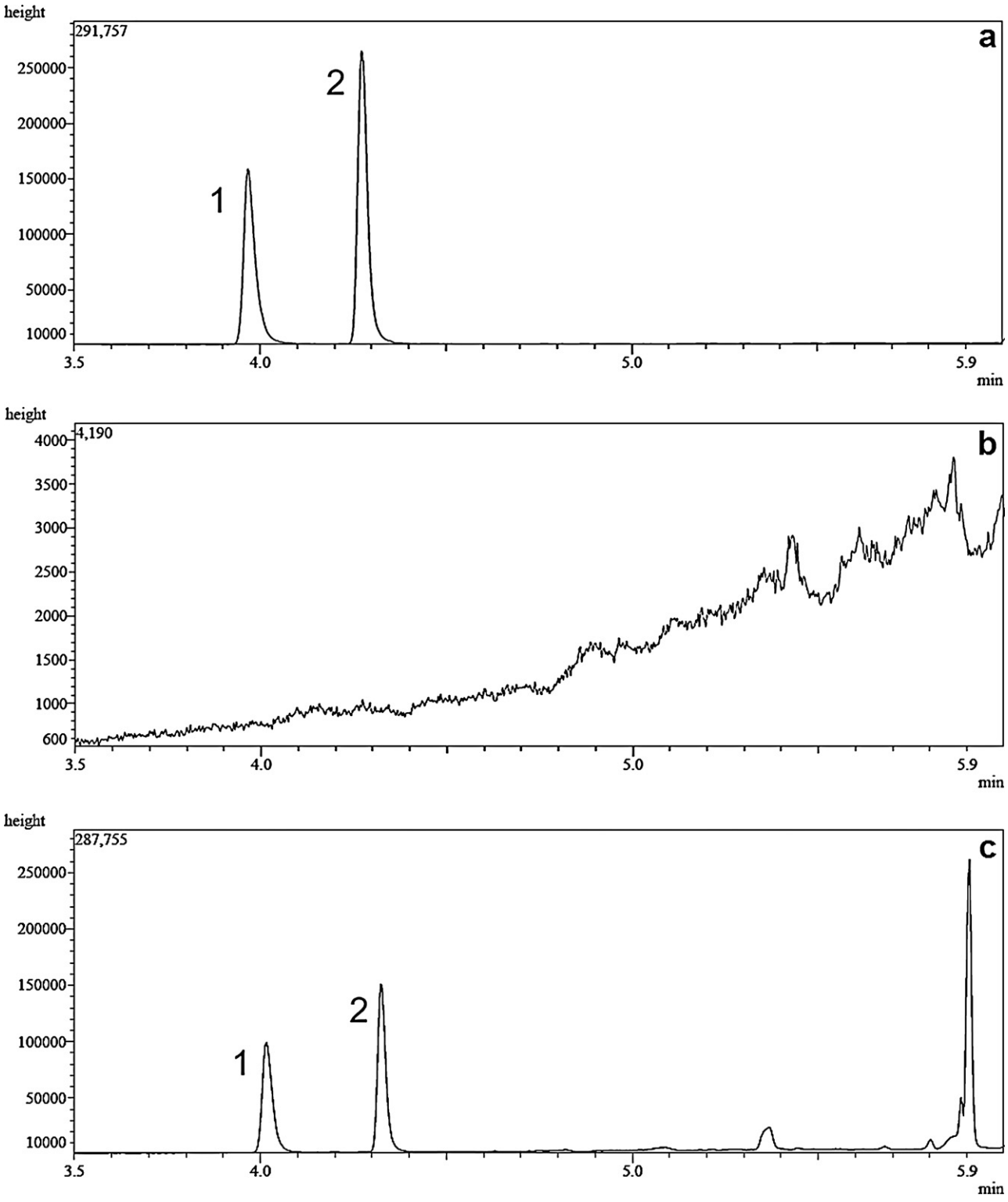

Fig. 5. Chromatograms obtained after MISPE optimization in the SIM mode (monitored using the ions at *m/z* 350 and *m/z* 366) for 500 μg L^{-1} of DETP and the DEDTP. (a) Aqueous standard, (b) blank urine sample and (c) urine sample fortified. (1) DETP and (2) DEDTP.

its detectability was enough to analyze the levels of dialkylphosphate commonly found in urine samples from individuals exposed to dissulfoton. In addition, within-day and between-day precision and accuracy produced acceptable relative standard deviations and relative errors, as demonstrated in Table 2. Three different cartridges were used, concomitantly, during all the optimization and validation steps, and non-significant differences were observed in the analytical signal (RSD < 5%). The lifetime of each cartridge was estimated in ca. 150 cycles, maintaining the same sensitivity and selectivity. The robustness assessment, through changes made in the nominal conditions, reflects the changes that can occur when a method is transferred to other laboratories, analysts, or equipment [36]. In this context, the Youden approach [36] allowed us to assess the robustness of the method and noted the influence

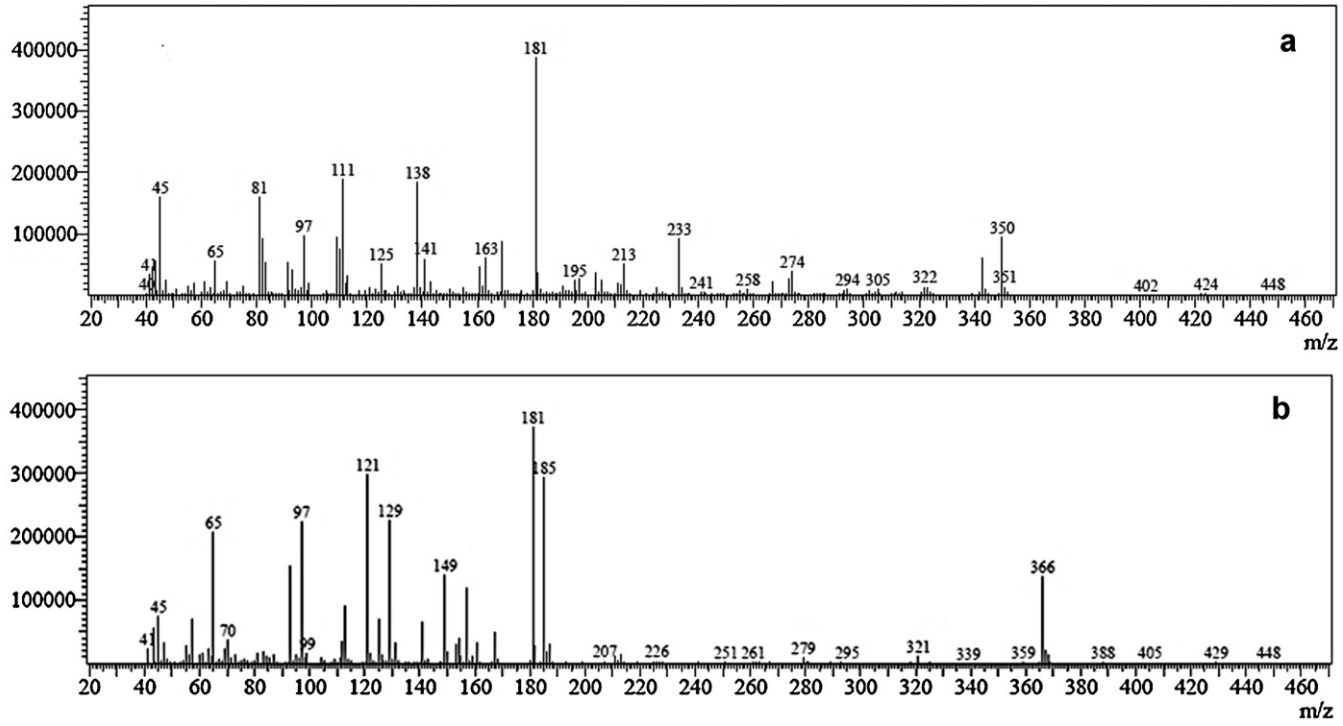

Fig. 6. Mass spectra obtained in the SCAN mode for DETP (a) and DEDTP (b).

Table 4
Effects of the analytical parameters used to evaluate the robustness in the DETP and DEDTP concentrations.

Analytical parameters	Parameter value in the resulting DETP (%)	Parameter value in the resulting DEDTP (%)
Injector temperature (°C)	−1.3	5.5
Source temperature (°C)	3.4	3.4
Interface temperature (°C)	7.5	4.0
Total flow rate (mL min^{-1})	5.4	1.2
Sample pH	1.7	3.3
Elution volume (mL)	0.5	3.7
Derivatization reagent volume (mL)	3.8	3.1

of each parameter in the final result. The variables evaluated are described in Table 3, which also demonstrates the factorial combination for assay and the formula to evaluate the variation effect. The results are presented in Table 4. All the variables evaluated showed an influence in the ±10% range, attesting to the robustness of the method. Fortified human urine samples (at 75, 150, 250, 400 and 500 μg L^{-1}) were analyzed by the proposed method and the results showed a very low variation between nominal concentration and analyzed concentration with relative error ranging from −5.7% to 2.64%.

4. Conclusions

The developed chromatographic method involving molecularly imprinted solid-phase extraction and GC–MS was appropriate for the analysis of DETP and DEDTP in urine samples. Good figures of merit were attained, such as low LOQ, wide linear range and good precision and accuracy. Additionally, other characteristics of this method should be emphasized, such as its high selectivity, high MIP cartridge lifetime, use of small urine volumes, possibility of MISPE mechanization and implementation for routine DAPs monitoring in occupational exposure to organophosphate pesticides.

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References

[1] P.G. Bardin, S.F. van Eeden, J.A. Moolman, A.P. Foden, J.R. Joubert, Ann. Intern. Med. 154 (1994) 1433.
[2] M. Maroni, A. Fait, Toxicology 78 (1993) 1975.
[3] C. Wu, P. Liu, L. Zheng, J. Chen, Z. Zhou, J. Chromatogr. B 878 (2010) 2575.
[4] R. Bravo, W.J. Driskell, R.D. Whitehead Jr., L.L. Needham, D.B. Barr, J. Anal. Toxicol. 26 (2002) 245.
[5] G.K.H. De Alwis, L.L. Needham, D.B. Barr, Talanta 77 (2009) 1063.
[6] D.E. Bradway, R. Moseman, R. May, Bull. Environ. Contam. Toxicol. 26 (1981) 520.
[7] Z. Vasilic, V. Drevenkar, V. Rumenjak, B. Stengl, Z. Frobe, Arch. Environ. Contam. Toxicol. 22 (1992) 351.
[8] T. Shafik, D.E. Bradway, H.F. Enos, A.R. Yobs, J. Agric. Food Chem. 21 (1973) 625.
[9] J. Hardt, J. Angerer, J. Anal. Toxicol. 24 (2000) 678.
[10] S.S. Park, H. Pyo, K.-J. Lee, S.-J. Park, Bull. Korean Chem. Soc. 19 (1998) 45.
[11] S.G. Wang, C.A. Franklin, R.C. Honeycutt, J.C. Reinert, J. Am. Chem. Soc. 16 (1989) 206.
[12] C.D. Brokopp, J.L. Wyatt, J. Gabica, Bull. Environ. Contam. Toxicol. 26 (1981) 524.
[13] A. Miki, H. Tsuchihashi, K. Ueda, M.Y. Yamashita, J. Chromatogr. A 718 (1995) 383.
[14] C. Aprea, G. Sciarra, L. Lunghini, J. Anal. Toxicol. 20 (1996) 559.
[15] T.F. Moate, C. Lu, R.A. Fenske, R.M. Hahne, D.A. Kalman, J. Anal. Toxicol. 23 (1999) 230.
[16] W.J. Driskell, M. Shih, L.L. Needham, D.B. Barr, J. Anal. Toxicol. 26 (2002) 6.
[17] J.E. Davies, J.C. Peterson, Ann. N.Y. Acad. Sci. 837 (1997) 257.
[18] N. Kupfermann, H. Schmoldt, J. Steinhart, J. Anal. Toxicol. 28 (2004) 242.
[19] A.N. Oglobline, G.E. O'Donnell, R. Geyer, G.M. Holder, B. Tattam, J. Anal. Toxicol. 25 (2001) 153.
[20] R. Bravo, L.M. Caltabiano, G. Weerasekera, R.D. Whitehead, C. Fernandez, L.L. Needham, A. Bradman, D.B. Barr, J. Expo. Anal. Environ. Epidemiol. 14 (2004) 249.
[21] K. Mosbach, O. Ramstrom, Nat. Biotechnol. 14 (1996) 163.

[22] C.S. Magalhães, J.S. Garcia, A.S. Lopes, E.C. Figueiredo, M.A.Z. Arruda, in: M.A.Z. Arruda (Ed.), Trends in Sample Preparation, Nova Science Publishers, New York, 2007, p. 245.
[23] D. Ortelli, S. Rudaz, S. Souverain, J.-L. Veuthey, J. Sep. Sci. 25 (2002) 222.
[24] S.G. Hu, L. Li, X.W. He, Anal. Chim. Acta 537 (2005) 215.
[25] A.G. Mayes, M.J. Whitcombe, Adv. Drug. Deliv. Rev. 57 (2005) 1742.
[26] N. Masqué, R.M. Marcé, F. Borrull, Trends Anal. Chem. 20 (2001) 477.
[27] E. Caro, R.M. Marcé, F. Borrull, P.A.G. Cormack, D.C. Sherrington, Trends Anal. Chem. 25 (2006) 143.
[28] E.C. Figueiredo, M.A.Z. Arruda, Br. J. Anal. Chem. 1 (2010) 36.
[29] E.C. Figueiredo, C.R.T. Tarley, L.T. Kubota, S. Rath, M.A.Z. Arruda, Microchem. J. 85 (2007) 290.
[30] R.V. Vitor, M.C.G. Martins, E.C. Figueiredo, I. Martins, Anal. Bioanal. Chem. 400 (2011) 2109.
[31] X. Hu, Y. Hu, G. Li, J. Chromatogr. A 1147 (2007) 1.
[32] J. He, R. Lv, H. Zhan, H. Wang, J. Cheng, K. Lu, F. Wang, Anal. Chim. Acta 674 (2010) 53.
[33] O.S. Amuda, A.A. Giwa, I.A. Bello, Biochem. Eng. J. 36 (2007) 174.
[34] M. Radhika, K. Palanivelu, J. Hazard. Mater. 138 (2006) 116.
[35] J.E. Barreiro, C.A.M. Fraga, Química medicinal: as bases moleculares da ação dos fármacos, 1st ed., Artmed, Porto Alegre, 2001.
[36] W.J. Youden, E.H. Steiner, Statistical Manual of AOAC, Association of Official Analytical Chemistry, Washington, USA, 1975.