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Mix-mode TiO-C_{18} Monolith Spin Column Extraction and GC-MS for the Simultaneous Assay of Organophosphorus Compounds and Glufosinate, and Glyphosate in Human Serum and Urine

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A rapid, specific, and sensitive method for the simultaneous quantitation of organophosphates (fenitrothion (MEP), malathion, and phenthoate (PAP)), glufosinate (GLUF), and glyphosate (GLYP) in human serum and urine by gas chromatography–mass spectrometry (GC-MS) has been validated. All of the targeted compounds together with the internal standard were extracted from the serum and urine using a mix-mode TiO-C_{18} monolithic spin column. The recovery of organophosphates from serum and urine ranged from 12.7 to 49.5%. The recovery of GLUF and GLYP from serum and urine ranged from 1.9 to 7.9%. The intra- and inter-accuracy and precision (expressed as relative standard deviation, %RSD) were within 96.7 – 107.7% and 4.0 – 13.8%, respectively. The detection and quantitation limits for serum and urine were 0.1 and 0.1 μg/ml, respectively, for organophosphates, 0.1 and 0.5 μg/ml, respectively for GLUF and GLYP. The method had linear calibration curves ranging from 0.1 to 25.0 μg/ml for organophosphates and 0.5 – 100.0 μg/ml for GLUF, and GLYP. The validated method was successfully applied to a clinical GLYP poisoning case.

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Introduction

Rapid analytical systems are very essential in clinical chemistry, because they enable timely and precise diagnosis and appropriate treatment. Rapid sample preparation prior to chromatographic analysis, in general, has numerous merits, and has long been a well-recognized direction in chromatographic technology. Recently, the monolithic spin column extraction method has been reported as being a quick extraction technique for use prior to chromatographic analysis.[1-3] Target compounds in a matrix are extracted using the monolithic spin column with only centrifugation. The eluting solvent is injected directly into the analytical instrument without the need for drying, because the final volume of the eluting solvent is small. Moreover, the process is completed in a short time, even if drying is necessary.[4]

Organophosphate insecticides are used worldwide, and insecticide poisoning has been known to occur in various settings. Fenitrothion (MEP) is the most commonly used organophosphate insecticide in Japan. The time-course patterns of the serum concentrations of MEP have been determined for various durations of hospitalization.[5] In this study, the serum MEP concentration of patients upon arrival at the hospital was reported to range from 3 to 12 μg/ml. Many analytical methods have been used for the assay of organophosphates, including GC-MS,[6] LC-MS,[7] and LC-diode-array detector (LC-DAD).[8]

Glufosinate (DL-homoalanin-4-yl-(methyl)phosphinate ammonium salt, GLUF) and glyphosate (*N*-(phosphonomethyl)glycine, GLYP) are phosphonic and amino acid group-containing chemicals that constitute an important category of pesticides, which are extensively used as herbicides in many countries, including Japan. Many cases of accidental and suicidal poisoning by the ingestion of these herbicides have been reported.[9-12] GLUF and GLYP have been assayed using a variety of analytical methods, including GC-MS[13-16] and LC-MS.[17] In patients with GLUF or GLYP poisoning, the blood GLUF or GLYP concentration ranges from 6.9 to 664.7 μg/ml.[12,14,16]

Relative to the total number of patients transported to our hospital for the treatment of substance overdose, such as antidepressants and/or antipsychotics, the number of patients experiencing pesticide poisoning has decreased by contrast. Although pesticide is less frequently analyzed, screening and/or quantity analyses are more important in the case of pesticide poisoning than the quantity analysis of antidepressants and/or antipsychotics.

In the case of impaired consciousness induced by prescribed medications, a cause can be surmised in a comparatively short

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time based on personal medicinal history. This, however, is not the case for pesticide poisoning in which early identification of the causative compounds is especially important. In the event of organophosphate, GLUF, and GLYP poisoning cases, quick detection of the herbicide that has been ingested is crucial. The simultaneous detection of these insecticides can help in providing appropriate medical treatment. In particular, the simultaneous analysis of different polar compounds is attractive with regard to the rapid determination of unknown compounds and/or in limited sample volumes.

Recently, we have reported the simultaneous analysis of diquat, paraquat, and MEP using MonoSpin C_{18} and GC-MS.[18] However, GLUF and GLYP cannot be extracted using a C_{18} column, because GLUF and GLYP are water-soluble compounds. Although we have reported the simultaneous analysis of GLUF and GLYP using a Sep-Pak® Plus PS-2 cartridge and GC-MS,[16] the process of solvent evaporation is time consuming. Moreover, organophosphate cannot be extracted using this cartridge. Thus, the simultaneous extraction of lipophilic organophosphates, such as MEP and water-soluble GLUF and GLYP from low-volume samples, is particularly challenging. Thus, MonoSpin C_{18} needs further improvement from a sorbent.

Water-soluble organic phosphates are strongly adsorbed on a titania (TiO_2) column.[19] Therefore, we have prepared a mix-mode TiO-C_{18} monolith spin column, and in this paper, a method for the simultaneous extraction of organophosphate, GLUF, and GLYP is presented using the mix-mode TiO-C_{18} monolithic spin columns. Although many types of organophosphate insecticides are commercially available in Japan, only certain types of compounds have been detected in our hospital. We selected MEP, malathion and phenthoate (PAP) as model organophosphate in this study. In our hospital, these compounds, which are commonly used in Japan, have often been detected in patients with organophosphate poisoning. The usefulness of the simultaneous extraction arises from the specific advantage of a mix-mode column.

Experimental

Chemicals

Mix-mode TiO-C_{18} monolithic spin columns were supplied by GL Sciences Inc. (Saitama, Japan). MEP, GLYP, malathion, and PAP were obtained from Wako Pure Chemical Industries (Osaka, Japan). GLUF was purchased from AccuStandard (New Haven, CT).

DL-2-Amino-3-phosphonopropionic acid (APPA) was purchased from Sigma (St. Louis, MO) and used as the internal standard (IS) for GLUF and GLYP. Fenitrothion-d_6 (MEP-d_6), used as an IS for MEP, was purchased from Hayashi Pure Chemical (Osaka, Japan). Acetic acid, 25% ammonia solution, and acetonitrile were purchased from Wako Pure Chemical Industries (Japan). The derivatization reagent *N*-methyl-*N*-(*tert*-butyldimethylsilyl) trifluoroacetamide (MTBSTFA) + 1% *tert*-butyldimethylchlorosilane (TBDMCS) was purchased from Pierce (Rockford, IL).

Standard solutions

GLUF and GLYP (10 mg/ml) were prepared in 10% methanol. These solutions were further diluted with 10% methanol to concentrations of 1000, 100 and 10 μg/ml and stored for a maximum of 3 months at 4°C, and used as calibrants. A stock solution of APPA (1 mg/ml) was prepared in 10% methanol and used as an IS for GLUF and GLYP analysis. The IS stock solution was further diluted with 10% methanol to a concentration of 100 μg/ml. Separate stock solutions of MEP, MEP-d_6, malathion, and PAP (10 mg/ml) in methanol were stored at –30°C. Calibrants were prepared from stock solutions, and were diluted to final concentrations of 1000, 100, and 10 μg/ml. The IS working solution of MEP-d_6 was prepared at a final concentration of 100 μg/ml. Quality-control (QC) samples of 0.1 (QC1), 1.0 (QC2), 7.5 (QC3), and 20 (QC4) μg/ml for MEP, malathion, and PAP were prepared separately. Similarly, QC samples of 0.5 (QC1), 3 (QC2), 30 (QC3), and 90 (QC4) μg/ml for GLYP and GLUF were prepared separately.

Calibration curve and quality control samples

Blank serum and urine samples (0.2 ml) were spiked with each of the compounds, to give concentrations of 0.1, 0.5, 1, 5, 10, and 25 μg/ml (MEP, malathion, and PAP) and 0.5, 1, 10, 25, 50, and 100 μg/ml (GLUF and GLYP) with the appropriate IS solution. The validation was done in six series of experiments.

Each quality-control (QC) solution was prepared in the same manner as mentioned above. QC samples for organophosphates were prepared using blank serum and urine samples (0.2 ml) containing 0.02 (QC1: 0.1 μg/ml), 0.2 (QC2: 1 μg/ml), 1.5 (QC2: 7.5 μg/ml), and 4 (QC4: 20 μg/ml) μg of each organophosphate. The quality-control solutions for GLUF and GLYP contained 0.01 (QC1: 0.5 μg/ml), 0.6 (QC2: 3 μg/ml), 6 (QC3: 30 μg/ml), and 18 (QC4: 90 μg/ml) μg of the compounds.

Extraction

First, 0.2 ml of water, 50 μl of acetic acid, and 10 μl (100 μg/ml) of both IS solutions were added to 0.2 ml of the serum and urine samples and vortexed for 15 s. This sample was then used for subsequent extraction.

The mix-mode TiO-C_{18} monolithic spin column was conditioned with 0.4 ml of acetonitrile at 3000 rpm for 1 min, followed by 0.4 ml of water at 3000 rpm for 1 min. The samples were applied to the conditioned monolith spin column. The column was centrifuged for 1 min at 3000 rpm. The column was subsequently washed with 0.2 ml water for 1 min at 3000 rpm. Analytes were eluted using 0.2 ml of a mixture of acetonitrile and 25% ammonia solution (9:1, v/v), for 1 min at 3000 rpm. The extract was evaporated to dryness under a stream of nitrogen at 45°C, in a heating block. To the residue was added 25 μl each of MTBSTFA with 1% TBDMCS and acetonitrile. After vortexing for 15 s, 1 μl of the derivatized sample was injected into the GC-MS system.

GC-MS analysis

Gas-chromatographic analyses were performed using an Agilent 6890 quadrupole gas chromatograph (GC) (Palo Alto, CA) equipped with an Agilent 5975B mass spectrometer (MS). A 30 m × 0.25 mm HP-5MS fused-silica capillary column with a film thickness of 0.25 μm, obtained from Agilent, was used. Helium (purity ≥ 99.999%) was used as a carrier gas at a flow rate of 0.6 ml/min. A 1-μl extract was injected in a splitless mode at an injection temperature of 250°C. The oven temperature was programmed to increase from an initial temperature of 100°C (held for 3 min) to 300°C (held for 3 min) at a rate of 20°C/min. The temperatures of the quadrupole, ion source, and mass-selective detector interface were 150, 230, and 280°C, respectively. The GC-MS system was operated in the selected-ion monitoring (SIM) mode with the electron multiplier tune value. The following ions were monitored (with quantitative ions in parentheses): MEP-d_6 (283.1), 266.0; MEP (277.0), 260.0; malathion (173.1), 158.0; PAP (274.0), 125.0; GLYP (454.2), 352.2; GLUF (466.3), 364.2; APPA (568.3), 466.3.

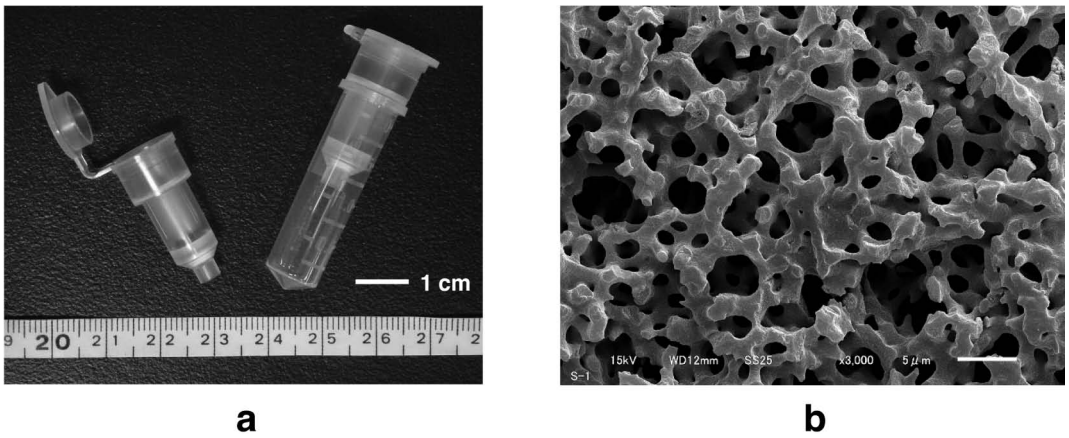

Fig. 1 Monolithic spin column (a), and the scanning electron microscope (SEM) image of the typical structure of monolith (b).

Method validation

The method was validated for selectivity, sensitivity, linearity, precision, accuracy, and recovery according to a method published on-line by the Food and Drug Administration (FDA).[20]

Limit of detection, limit of quantitation, and carry-over

The limit of detection (LOD) and lower limit of quantitation (LLOQ: QC1) were determined by a method recommended by the FDA.[20] Six different blanks and six different spiked serum and urine samples at QC1 levels were analyzed in three repetitions over a period of three days. The carry-over was measured by running a blank serum and urine sample after a spiked serum and urine sample (QC1) in three repetitions. The area ratios had to be less than 1%, and the concentrations in the blank serum and urine samples had to be less than 20% of the determined QC1.

Accuracy and precision

The concentrations of the QC samples were selected to encompass the whole range of the calibration curve corresponding to the compound levels anticipated to occur in most patient samples: QC1, QC2, QC3 and QC4. The concentration selected for the QC2 sample corresponds to 3-times the respective lower limit of quantification (*i.e*. the lowest calibration level) kept in the finalized method, in accordance with the FDA recommendations.[20] Replicate analysis ($n = 6$) of four QC samples was used for intra-assay precision and accuracy determination. The inter-assay accuracy and precision were determined by duplicate analysis of the four QC samples repeated on three different days. The calculated precision was expressed as the relative standard deviation (%RSD), and the accuracy was calculated as the bias or percentage of deviation between the nominal and measured concentrations. Precision values of less than 15% and accuracy values within the range of 85 – 115% determined the measurement range, except for the lower limit of quantification (QC1), which was the level that had a precision of less than 20% and an accuracy of between 80 and 120%. After completion of the above validation procedure, duplicate QC samples at the four concentration levels (QC1, QC2, QC3 and QC4) were used for the routine analysis of patient samples. Precision and accuracy investigations also validated the applied standard curve fit.

Recovery

Recoveries were estimated from the un-extraction and extraction areas of the analytes and ISs. Blank serum, urine with or without analyte and IS were extracted in a similar manner. The eluent was spiked with analyte and IS at concentrations corresponding to serum and urine in the three QC levels. The spiked eluent was evaporated and derivatized, and subsequently analyzed using GC-MS (un-extraction). The spiked serum and urine samples were extracted and derivatized, followed by analysis in a similar manner (extraction). The un-extraction areas of the analytes were compared with the extraction areas the corresponding levels.

Application

The proposed GC-MS method was applied to an actual case of poisoning. An 86-year-old female was found lying in her house. One bottle of GLYP (GLYP, 41%, 500 ml) was found near her body. The maximum estimated ingested amount was 100 ml, upon her arrival at our emergency department. Ten days later, the subject was discharged, fully recovered after a suicide attempt. Toxicological analyses were performed on her serum and urine samples. A collected blood sample was immediately centrifuged at 3000*g* for 10 min, and was subsequently analyzed.

Results and Discussion

Morphology and specificity

Figure 1 shows the monolithic spin column and the typical structure of the monolith. Figures 2, 3, and 4 show representative chromatograms of blank human serum and urine; the QC1 of MEP, malathion, PAP, GLUF, and GLYP with ISs in blank serum and urine, and the poisoning patient's serum and urine sample. Typical retention times for MEP-d_6, MEP, malathion, PAP, GLUF, GLYP, and APPA were 10.54, 10.57, 10.58, 11.24, 12.69, 13.11, and 13.48 min, respectively. The chromatograms produced clean extracts, with no interference from endogenous compounds, at the retention times for MEP, malathion, PAP, GLUF, GLYP, and the ISs.

Extraction

Phosphate-containing compounds are readily adsorbed onto titania coated silica surfaces under acidic conditions.[21] Therefore, the first step in the optimization of the spin column extraction procedure was evaluated; 0.2 ml aliquots of serum and urine samples were added to acetic acid (10, 25, 50, and 100 μl) and 0.2 ml water. This sample was applied to the

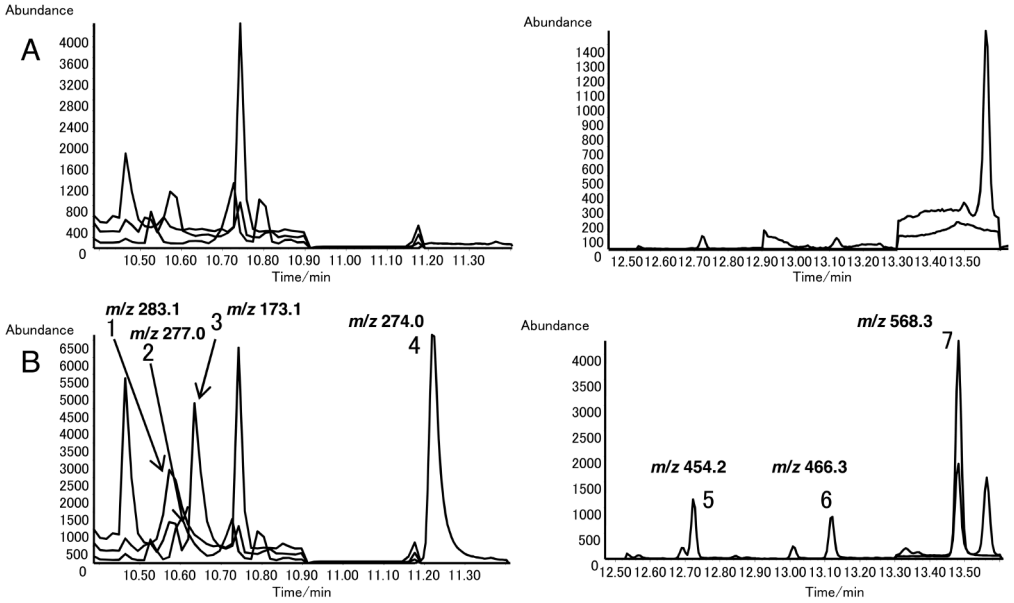

Fig. 2 Typical SIM chromatograms of blank serum (A); blank serum with a QC1 (0.1 and 0.5 μg/ml) and with IS. 1, MEP-d_6; 2, MEP; 3, malathion; 4, PAP; 5, GLYP; 6, GLUF; 7, APPA (B).

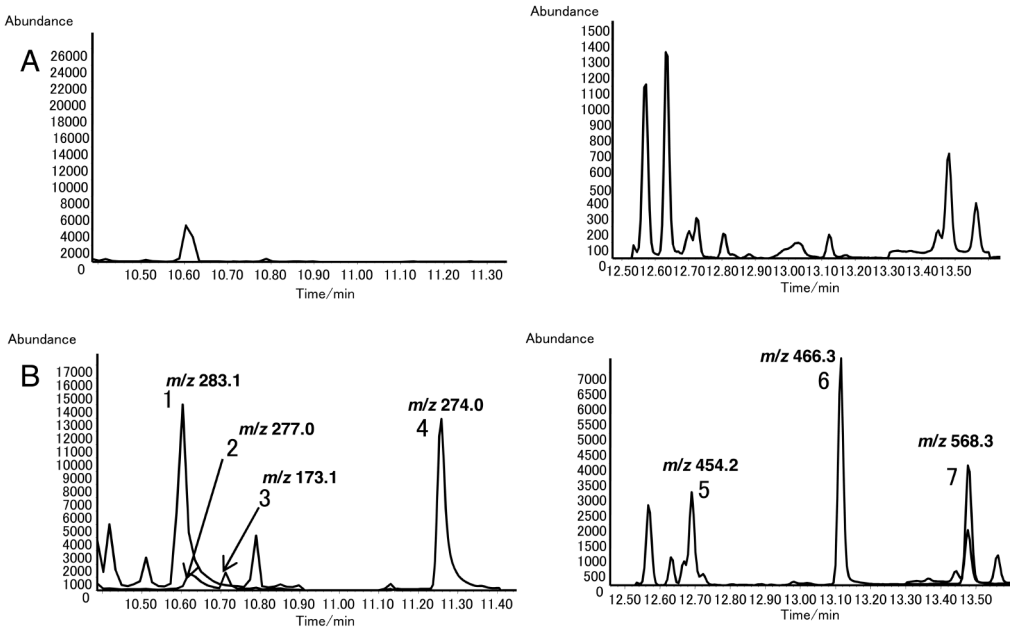

Fig. 3 Typical SIM chromatograms of blank urine (A); blank urine with a QC1 (0.1 and 0.5 μg/ml) and with IS. 1, MEP-d_6; 2, MEP; 3, malathion; 4, PAP; 5, GLYP; 6, GLUF; 7, APPA (B).

conditioned monolith spin column. The spin column was then washed with water and eluted with acetonitrile. However, not all of the compounds were detected, and thus small amounts of NH_4OH were needed to fully elute the compounds from the monolith. Optimal results were obtained with the addition of 50 μl of acetic acid, and the maximum recovery at this level of efficiency was obtained using an acetonitrile:NH_4OH (9:1) eluent.

Internal standard

The compound used as the IS must be a stable analyte in order to surmount sample matrix effects. In fact, the use of MEP-d_6 as an IS for organophosphates in the proposed method appears to effectively negate most of the residual relative matrix effect variability. However, because a suitable IS for GLUF and GLYP was not commercially available, an alternative approach was undertaken. The chosen IS should match the chromatographic properties, recovery and ionization properties of the analyte.[22] APPA matched these criteria, and also served our purpose of method development; therefore, it was chosen as an IS for GLUF and GLYP. APPA contains the –OH, –COOH and $–NH_2$ functional groups, and is easily derivatized with MTBSTFA + 1% TBDMCS by vortexing at room temperature.

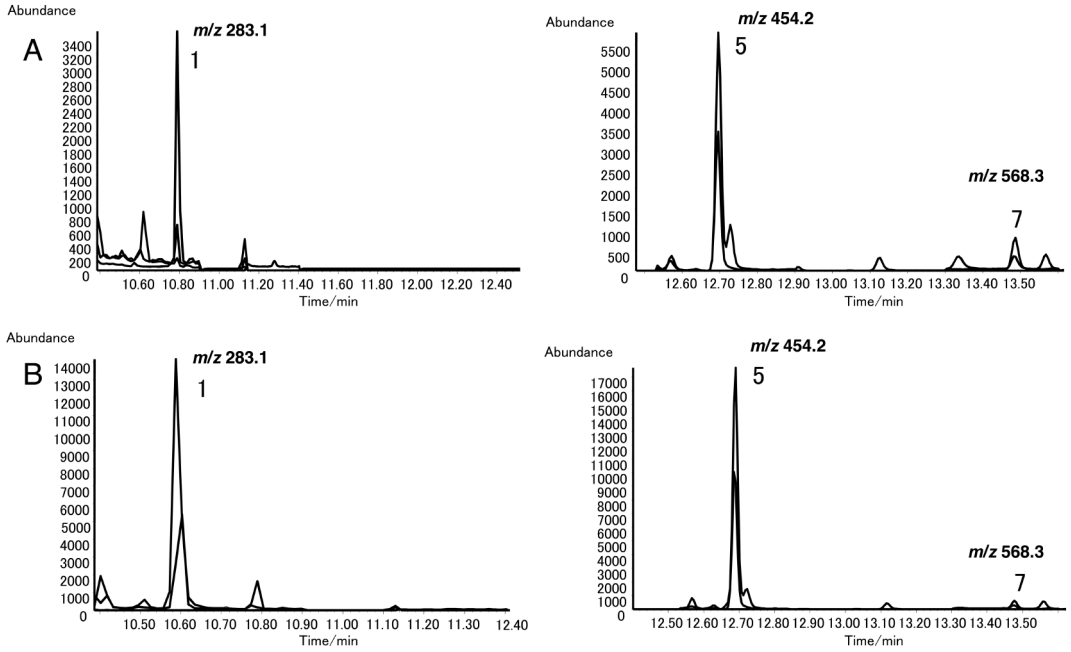

Fig. 4 Serum (A) and urine (B) samples obtained from a poisoning patient 4 and 10.5 h after oral ingestion of GLYP. 1, MEP-d_6; 5, GLYP; 7, APPA.

Table 1 Limit of detection (LOD) and linearity

	LOD/μg ml^{-1}	Linearity/μg ml^{-1}	Regression equation
Serum			
Fenitrothion	0.1	0.1 - 25	$y = 0.3851x - 0.3791$
Malathion	0.1	0.1 - 25	$y = 0.9642x + 0.6675$
Phenthoate	0.1	0.1 - 25	$y = 0.8246x + 0.2439$
Glufosinate	0.1	0.5 - 100	$y = 0.2375x + 0.2477$
Glyphosate	0.1	0.5 - 100	$y = 0.0192x - 0.2324$
Urine			
Fenitrothion	0.1	0.1 - 25	$y = 0.3557x - 0.0781$
Malathion	0.1	0.1 - 25	$y = 0.3139x - 0.3885$
Phenthoate	0.1	0.1 - 25	$y = 0.9979x - 0.0768$
Glufosinate	0.1	0.5 - 100	$y = 0.1682x - 0.0489$
Glyphosate	0.1	0.5 - 100	$y = 0.0284x + 0.0969$

Selectivity

No peaks corresponding to endogenous compounds were observed at the retention times of the analytes in any of the blank serum and urine extracts (Figs. 2 and 3). The ion selected for monitoring was chosen based on its relative abundance, while avoiding possible structural analogies with the other analyzed compounds.

LOD and LOQ

The determined LODs and LLOQs are listed in Table 1. The LOD of MEP, malathion, PAP, GLUF, and GLYP was 0.1 μg/ml in all cases. On the basis of the aforementioned criteria, the LLOQs of MEP, malathion, PAP, GLUF, and GLYP were determined to be 0.1, 0.1, 0.1, 0.5, and 0.5 μg/ml, respectively, when 0.2 ml serum and urine samples were used.

Calibration curve

Calibration curves over the entire range of concentrations delineated in Table 1 were satisfactorily described by a linear relationship of the peak-area ratio of each compound to its IS, *versus* the concentrations of the respective analytes in each standard sample. The determination coefficients (r^2) of all of the calibration curves were higher than 0.999 with back-calculated concentrations of the calibration samples falling within ±15% of the nominal values (±20% at LLOQ). Of note, the chosen calibration ranges were initially selected to cover the clinical range of organophosphate, GLUF, and GLYP concentrations previously reported in the literature.[11,13-15]

Precision and accuracy

The precision and accuracy determined with the QC1, QC2, QC3, and QC4 samples are summarized in Table 2. The intra-day precision of the method was calculated as the relative standard deviation (RSD) of the assays for intra-day accuracy. The inter-day precision of this method was expressed as the RSD of the assays for the inter-day accuracy. The mean intra-assay precision was similar over the entire concentration range, and was less than 13.8% in all cases. Overall, the mean inter-day precision was within 4.9 and 13.6%.

Matrix effects and recovery

No noticeable matrix effects (no drifts or shifts of the signals) were observed at the respective retention times of the organophosphates, GLUF, and GLYP analytes and the ISs used. The blank serum or the urine matrix does not appear to significantly interfere with the target compounds and the ISs.

The purity of the coating material (*i.e.* how much titania is contained in the silica) will also dictate whether water-soluble organic phosphates will adhered to the monolithic column. Miyazaki *et al.*[23] reported the preparation of titania-coated monolithic silica based packing materials for HPLC columns using a sol-gel method, and investigated their properties. The monolithic spin column used in this study was basically prepared according to their preparation method.

Previously, we reported an analytical method for assaying GLUF and GLYP that employed protein precipitation prior

Table 2 Precision, accuracy and extraction recovery of organophosphates, GLUF, and GLYP from the serum and urine quality control (QC) samples

	Spiked conc./ μg ml^{-1}	Intra-day		Inter-day		
		Accuracy, %[a]	RSD, %	Accuracy, %[b]	RSD, %	Recovery, %[c]
Serum						
Fenitrothion	0.1	97.8	10.9	98.0	8.5	14.3 ± 2.7
	1	105.3	11.9	102.9	11.7	12.7 ± 3.0
	7.5	102.9	11.2	99.4	10.2	18.5 ± 2.3
	20	101.2	6.8	101.4	7.6	20.0 ± 3.6
Malathion	0.1	100.0	5.6	100.0	6.0	47.9 ± 3.7
	1	107.7	13.5	105.8	13.5	49.5 ± 1.2
	7.5	98.9	12.6	99.2	13.6	40.9 ± 4.5
	20	101.9	4.0	104.0	6.7	39.7 ± 4.3
Phenthoate	0.1	101.0	4.5	100.7	4.9	29.2 ± 2.6
	1	104.3	10.8	104.9	11.2	23.4 ± 3.2
	7.5	102.4	9.0	101.0	8.1	25.7 ± 1.0
	20	101.1	9.2	101.8	7.6	27.8 ± 3.5
Glufosinate	0.5	98.3	10.9	97.2	9.7	4.1 ± 1.5
	3	106.2	13.0	104.8	12.3	4.7 ± 1.8
	30	102.1	9.9	101.9	8.4	6.0 ± 1.7
	90	103.8	11.1	101.0	10.4	7.9 ± 1.1
Glyphosate	0.5	96.7	9.5	98.1	11.5	3.6 ± 1.1
	3	102.8	13.8	100.0	12.6	2.4 ± 0.9
	30	101.9	8.1	100.6	7.1	3.1 ± 0.8
	90	98.8	12.0	101.6	9.8	2.3 ± 0.9
Urine						
Fenitrothion	0.1	96.7	9.7	98.6	6.4	30.3 ± 2.6
	1	99.6	9.0	101.9	9.4	27.7 ± 4.0
	7.5	102.9	8.7	100.2	9.0	28.5 ± 2.3
	20	99.8	6.1	101.7	8.6	31.6 ± 2.7
Malathion	0.1	97.7	9.7	95.8	7.2	41.1 ± 2.8
	1	106.3	7.0	104.4	9.0	38.2 ± 3.5
	7.5	100.4	9.0	98.9	8.5	44.0 ± 2.4
	20	97.8	4.8	101.0	8.6	43.1 ± 4.5
Phenthoate	0.1	99.5	8.6	100.1	7.9	38.1 ± 3.0
	1	100.8	9.9	103.1	9.2	35.1 ± 2.8
	7.5	100.8	7.6	99.9	8.6	35.7 ± 1.0
	20	102.2	5.1	103.1	7.9	37.8 ± 4.6
Glufosinate	0.5	103.0	8.9	101.9	9.7	2.8 ± 0.4
	3	105.2	6.9	104.5	10.7	2.8 ± 0.8
	30	101.3	7.6	102.7	6.9	2.6 ± 0.6
	90	99.1	7.9	104.5	8.9	2.7 ± 0.7
Glyphosate	0.5	103.3	11.8	98.9	12.3	2.0 ± 0.3
	3	103.7	7.3	100.3	10.8	1.9 ± 0.4
	30	101.7	4.9	102.1	6.4	1.9 ± 0.2
	90	99.9	9.9	103.9	9.2	2.1 ± 0.5

a. Intra-day accuracy results were obtained from six replicate samples ($n = 6$) for each concentration of the analyte analyzed on a single day.
b. Inter-day accuracy results were measured using a minimum of six determinations per concentration of the analyte on three separate days.
c. Data are expressed as mean ± SD.

to the extraction process, after which approximately half of the sample was extracted using a solid-phase extraction technique.[16] The overall recoveries achieved using that method were 37.5 – 41.6%. However, the extraction recovery of the present method was very low, even without protein precipitation. As indicated in Table 2, the extraction recovery values were consistently lower than 7.9%. It has been found that the recoveries of phosphatidyl-ethanol-amine and L-α-phosphatidylcholine dipalmitoyl in egg yolk by titania extraction were 62.8 and 63.1%, respectively.[21] It is thus postulated that phospholipids may adhere to titania more readily than GLUF and GLYP. Moreover, all of the organic phosphates were adsorbed, while sugars, carboxylic acids and other compounds were not much adsorbed on titania.[19] In the TiO-C_{18} monolithic column used in this study, TiO and C_{18} were modified to 44 and 56%, respectively. The recovery of GLUF and GLYP increase, and that of organophosphates possibly decreased with the increase in the TiO rate. Moreover, the derivatization of TBDMCS was an extremely sensitive procedure. Detection was possible, although the recovery of GLUF and GLYP was very low. Most importantly, the proposed method has a very low extraction recovery, although the polarity of the compound extracted is different from that of the previous study.

Application

The validated method has been successfully applied to GLYP analysis in serum and urine samples. The serum and urine GLYP concentrations in samples obtained from a poisoning patient at 4 and 10.5 h after ingestion were determined at 13.2 and 4.5 μg/ml, respectively (Figs. 4A and 4B).

Method comparison

The proposed method was compared with previous methods comprised of GC-MS analysis with protein precipitation prior to solid-phase extraction.[14–16] Both the extraction and derivatization times of the present method and the previous methods were compared. In the present method, the sample-preparation process prior to injection into the GC-MS takes approximately 15 min, which presents a one-third reduction of the extraction time. Finally, the proposed method might be extended to the determination of other different polar compounds in biological materials.

Conclusions

This study describes a method for the simultaneously rapid extraction of organophosphates, GLUF, and GLYP from human serum and urine by using mixed-mode TiO-C_{18} monolithic spin columns. Using this method, 0.1 – 25 μg/ml of organophosphate and 0.5 – 100 μg/ml of GLUF and GLYP could be extracted. This method was reliable, selective, and accurate. Although the recoveries of GLUF and GLYP were very low, these compounds were satisfactorily detected after TBDMCS derivatization. The application of this method for detecting GLYP concentration in the serum and urine samples collected from a patient with GLYP poisoning confirmed the usefulness of this method.

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