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HPLC-UV platform for trace analysis of three isomeric mononitrophenols in water with chitin based solid phase extraction†

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A method is described for simultaneous determination of trace levels of three isomeric mononitrophenols (MNPs) in environmental water samples using chitin (cheap, effective, biocompatible, green and safe) based solid phase extraction and high-performance liquid chromatography-ultraviolet detection. Various parameters were investigated and optimized for extraction and enrichment of three MNPs, and chitin-MNP adsorption was investigated using SEM, FT-IR and Raman spectroscopy. Validation tests of spiked water samples showed good linearities for all three MNPs (R^2 = 0.9990–0.9999) over a wide concentration range of 0.001–1 μg mL^{-1}. Limits of detection (LODs) and quantification (LOQs) were measured at ng mL^{-1} levels: 0.13 ng mL^{-1} and 0.39 ng mL^{-1} for 2-NP, 0.09 ng mL^{-1} and 0.27 ng mL^{-1} for 3-NP and 0.19 ng mL^{-1} and 0.57 ng mL^{-1} for 4-NP. Inter- and intra-day precision tests showed variations of 1.67–1.80%, 1.06–1.56%, and 1.14–1.62% for 2-NP, 3-NP, and 4-NP, respectively. Average recoveries were in the range of 56.70–97.51%, with relative standard deviations below 15%. The developed method was then applied to analyze 8 real environmental water samples. Moreover, 4-NP was the most frequently detected MNP at concentration levels of 0.749–0.947 μg mL^{-1}, followed by 2-NP. Furthermore, 3-NP was not detected in any studied real sample. The validated method is simple and economical, with adequate sensitivity for trace levels of three isomeric MNPs. This method could be adopted by water quality analysis laboratories and allows an easy expansion to other related nitrophenols (NP) in water matrices' analysis.

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1. Introduction

Nitrophenols (NPs) are extensively used in petrochemical synthesis, including plastics, pesticides, paints, rubber, dyes, and pulp production.[1] In recent years, the presence of nitrophenols in the industrial wastewater has great concerns, due to their toxicity to the receiving bodies and the overall increase in industrial wastewater discharge to the environment.[2] The three isomeric mononitrophenols (MNPs), namely 2-nitrophenol (2-NP), 3-nitrophenol (3-NP), and 4-nitrophenol (4-NP) (Fig. 1A), are widely applied in the agricultural, pharmaceutical and chemical industries,[3] and during these application processes, some of them are inadvertently released into the environment, which in turn has led to the accumulation of mononitrophenols as contaminants in soil, rivers and ground waters. In environmental waters, a simple contact of nitrite ion with phenolic wastewaters under ambient conditions may also form mononitrophenols.[4] The MNPs may also be inadvertently produced by microbial or photodegradation of pesticides, which contain MNP moieties. MNPs are listed as priority toxic pollutants and hazardous wastes due to their toxic effect on plants, animals and humans, even at low concentrations.[5,6] In addition, the toxicity of these three isomers is different from each other: 2-NP and 4-NP present more adverse effects on the development and metabolism of an organism,[7] and 4-NP has an intense toxic effect on methaemoglobin formation, causing kidney and liver damage, systemic poisoning, anaemia, and skin and eye irritation.[8,9]

As a result of equal molecular weight and similar physiochemical properties of three isomeric MNPs, it is difficult to separate, identify and quantify them at trace levels and have provoked analytical researchers to innovate various methods to analyse them;[10] thus, determination of MNPs in water at trace levels is an important topic in the field of environmental science, water research and regulatory agencies and has attracted much attention in recent years.[11–15]

Owing to the trace concentrations of MNPs and much interference found in environmental water samples, the sample clean-up and enrichment steps to improve the method

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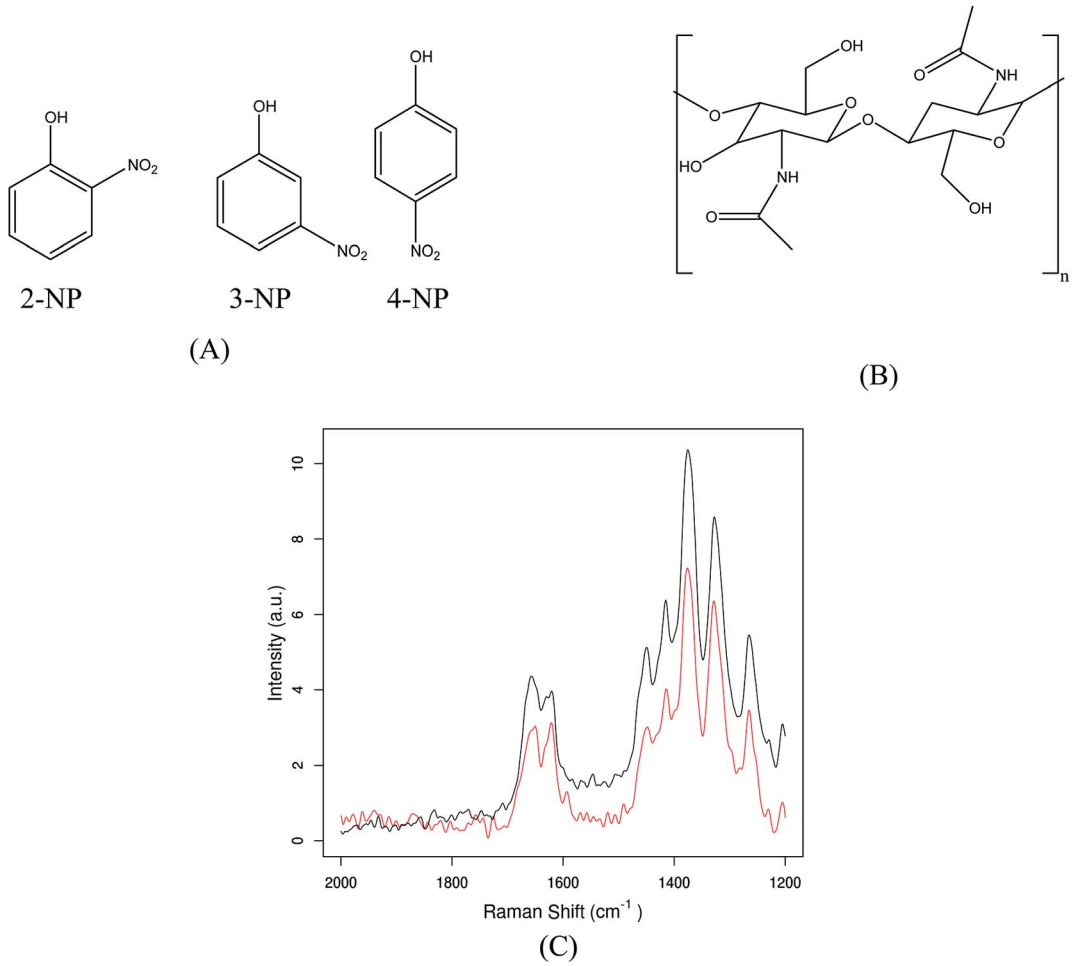

Fig. 1 Chemical structures of three MNPs (A) and chitin (B). (C) FT-Raman spectra of chitin (black) and chitin-*p*-nitrophenol loaded (red) powders in the range of 2000–1200 cm^{-1}.

sensitivity, recovery and accuracy are of paramount importance, prior to the analysis of MNPs. Among various water analysis and purification techniques, adsorption is a fast, inexpensive and widely applicable technique.[16] Moreover, it is universal in nature as it can be applied for the removal of soluble and insoluble contaminants and biological pollutants with a removal efficiency of 90–99%. Thus, the use and development of economic and green adsorbents has led to the rapid growth of research interests in the analytical field. Solid phase extraction (SPE) is a promising sample pretreatment technique owing to its ease of use, flexibility, short extraction time, safety, low consumption of organic solvents and high enrichment factor. It has been widely used for analysis of phenolic compounds.[17] The SPE always encourages green and efficient adsorbents with stronger adsorption, selectivity, and sensitivity of the method. Thus, the application of specific, green and efficient adsorbents for SPE of MNPs would be of particular significance, and in this study, we have developed an analytical protocol using chitin based SPE coupled with high-performance liquid chromatography-ultraviolet detection (HPLC-UV) for the trace analysis of three isomeric MNPs in water matrices. Chitin, the second most abundant natural biopolymer after cellulose, the main component of the cell walls of fungi, exoskeletons of arthropods and insects, is a long-chain polymer of *N*-acetylglucosamine (Fig. 1B) and is renewable, biodegradable, biocompatible and bio-absorbable with low immunogenicity and antibacterial properties with various applications in the field of agriculture, industry, medicine and biomedical research.[18–20] As an adsorbent, chitin is known for its high capacity to remove organic and inorganic contaminants from water.[21,22] Such versatility is due to the presence of acetamide groups (–CONH–), hydroxyl group (–OH) and bridged electronegative oxygen atom (O) on its structure as these groups have great ability for chemical and physical interaction with other species[21,23] and has been used for removal of metals, inorganic cations and anions.[24–27]

To the best knowledge of the authors, chitin based SPE is herein, for the first time, proposed and established for trace level, simultaneous determination of three isomeric MNPs. The primary objective of this study was to develop and validate a simple, fast, sensitive, green and reliable analytical method based on chitin based solid phase extraction procedure for the simultaneous identification and quantification of three isomeric MNPs using HPLC-UV. Special attention was paid to optimizing the SPE procedure and briefly investigating possible chitin-MNP

interactions. In addition, an extensive validation study was used to evaluate the analytical performance. The developed method was then assessed for its real-world applicability by analyzing three target MNPs in 8 different environmental water samples.

2. Experiment

2.1 Chemicals and materials

Chemicals were purchased from the following sources: three MNP analytical standards including 2-NP, 3-NP and 4-NP were purchased from the Sigma Aldrich (Mumbai India). They were stable over a period of at least six months. Methanol and acetonitrile (HPLC grade) were also obtained from Sigma Aldrich (Mumbai India). HPLC-grade water was from Merck (Mumbai India). Pure chitin was purchased from Aldrich (Germany). Chitin was crushed and passed through a sieve to coarse flake consistency. All other solvents used were of analytical grade.

2.2 Interaction assay

The mechanism of interaction of chitin with the nitrophenols was studied by means of FT-Raman spectroscopy. Interaction assay was carried out by a batch method. A high load of *p*-nitrophenol (10 mg) was incubated with chitin (40 mg) in ultrapure water (10 mL) for 24 h in a room with controlled temperature (25 °C) and constant stirring (120 rpm). Chitin was then centrifuged for 5 min at 10 000 rpm. Supernatant was discarded, and chitin was resuspended for 15 min in 10 mL of ultrapure water upon vigorous stirring. The centrifugation and resuspension steps were repeated 3 times. Finally, *p*-nitrophenol loaded chitin powder was dried at 37 °C for 16 h. This procedure was also performed for chitin powder without the addition of *p*-nitrophenol.

ATR-FTIR (diamond attenuated total reflectance) and FT-Raman spectra of chitin and *p*-nitrophenol loaded chitin powders were recorded using a Nicolet iS50 Advanced spectrometer (Thermo Scientific). ATR-FTIR spectra were recorded with 32 scans and a resolution of 1 cm^{-1}. FT-Raman spectra were acquired with an excitation laser beam of 1064 nm, 0.3 W laser power, resolution of 4 cm^{-1}, and 500 scans.

2.3 Preparation of standard solution

A stock solution of three mixed MNP standards were prepared in methanol at the concentration of 1 mg mL^{-1} and stored at −20 °C in a refrigerator. The standard working solutions were obtained daily by appropriate dilution of the stock solution with water.

2.4 Apparatus

The analysis of three MNPs was carried out using Dionex HPLC instrument. The instrument consists of a UVD170U UV/VIS detector, LC P680 pump, 20 μL sample loop, and LC chromeleon chromatography management software. Separation was performed using a Purospher® STAR C_{18} (250 × 4.6 mm, 5 μm) column from Merck Germany. The following parameters were set for quantitative determination of three MNPs in the eluates: the detector wavelength was 279 nm; 20 μL of the eluate was injected into the instrument; the mobile phase was (50MeOH : 50H_2O) at flow rate of 1.0 mL min^{-1}.

Solid phase extraction (SPE) of three MNPs was performed using a visiprep-12-port vacuum manifold from Supelco, Germany. The flow rate was controlled using vaccum pumping. The outlet tip of the manifold was connected to a vacuum pump (RIVOTEK, TID-25P, India). Chitin was packed in cartridges by placing specific mass of the adsorbent in an empty 3 mL polypropylene SPE-tube (Supelco, USA). A mortar was used for crushing the chitin to coarse flake consistency. Two test sieves (Aldrich, Germany), 8 mesh × 2.36 mm and 18 mesh × 1 mm, respectively, were used for particle size control of the sorbent.

2.5 Sampling and sample preparation

Two agricultural run-off water samples were collected from an agriculture field in the village Marara, Punjab, India. Two industrial wastewater samples were collected from the discharge site of Ludhiana pesticide manufacturing company and two river water samples from the Nalva River, Pathankot. Two tap water samples were collected from the research lab. Before use, all the collected samples were filtered through a simple Whattman paper 1, followed by 0.45 μm micropore filter membranes (Whatman, Germany) and stored in polyethylene bottles at 4 °C.

2.6 General SPE procedure

A cartridge was prepared by placing 100 mg of chitin in an empty 3 mL polypropylene SPE tube (Supelco, USA). Polyethylene frits of 20 μm porosity (Supelco) were used to hold the chitin packing in the cartridge. The cartridge was conditioned with 5 mL of distilled water, followed by 5 mL of methanol, before an extraction procedure. The SPE procedure was executed as follows: 5 mL of water sample was passed under the force of a vaccum pump through the sorbent at a controlled flow rate of 2 mL min^{-1}. Next, the cartridge was washed with 1 mL of distilled water and vacuum-dried for 3 minutes. The retained MNPs were eluted with 250 μL of acetonitrile. Finally, the eluted solution was filtered through a 0.22 μm syringe filter prior to injection into the HPLC-UV system for analysis.

3. Method validation

The optimum SPE and HPLC-UV conditions described above were used to determine the presence of any quantity target MNPs as a way to verify the real world applicability of the method. The validation procedure was performed following the International Conference on Harmonisation (ICH) guidelines. The analytical performance characteristics investigated included selectivity, calibration curve, ability to determine a linear calibration curve, the limits of detection (LOD) and quantification (LOQ), precision, and trueness (expressed as recovery rate). Precision was determined by measuring the relative standard deviation (RSD) and was evaluated as inter- and intra-day precision.

3.1 Selectivity

The selectivity of the proposed SPE method was verified by comparing results from a control (MNP-free) water sample (ESI

Fig. S3A†) and a sample fortified with three MNPs (Fig. 3A). As is shown in Fig. 3A and B, no interfering peaks were observed at the retention times of individual compounds, proving that there was sufficient selectivity for the analysis of multiple pesticides at trace levels. Fig. 3B also showed that the control samples did not give false-positive signals.

3.2 Calibration curve and linearity

Calibration curves were constructed using standard working solutions at eight concentration levels obtained by diluting the standard stock solution with distilled water (0.001, 0.005, 0.01, 0.05, 0.1, 0.4, 0.8 and 1.0 μg mL^{-1}) and then analyzed under previously described chromatographic conditions. A calibration method based on the peak area of each analyte was then used to quantify targets. A calibration curve in the form of $y = Ax + B$ was constructed by plotting the peak areas (y) against standard concentrations (x). The results shown in Table 1 demonstrated that calibration curves with excellent linearity were obtained for all three MNPs. The coefficients of determination (R) were higher than 0.99 in a wide concentration range for all targets.

3.3 LOD and LOQ

LODs were estimated using the minimum concentrations detected for all three MNPs based on signal-to-noise (S/N) ratio of 3, and LOQs were set as 10 times this ratio. As listed in Table 1, the LOD and LOQ values were found to be at the low ng mL^{-1} level, with LOD values 0.13 μg mL^{-1}, 0.09 μg mL^{-1}, and 0.19 μg mL^{-1} for 2-NP, 3-NP and 4-NP, respectively.

3.4 Precision and stability

To gauge the precision of the method, intra- and inter-day variations were estimated and expressed as RSD of the peak areas for each analyte following an analysis of 1 μg mL^{-1} standard working solution injected five times consecutively on the same day and injected five times over five consecutive days. The results in Table 1 show that inter-day variation of peak areas for three MNPs were in the range of 1.56–1.80% and intra-day variations of 1.06–1.67%. Stability was investigated by injecting a distilled water sample spiked with 10 μg mL^{-1} of three MNPs at 0, 2, 4, 6, 10, and 12 h. The RSD values were lower than 10%, as shown in Table 1. All of the above results indicated that the proposed method was precise and that the fortified samples were stable.

3.5 Trueness

The trueness of the developed method was determined through recovery studies using a sample of distilled water and two tap water samples fortified at three (high, medium and low) spiking levels, which were 0.005, 0.1, and 1 μg mL^{-1} for each of three MNPs. Next, all samples were extracted and analyzed in triplicate following the previously described procedure. The recovery percentages were then calculated using the following equation:

$$\text{Recovery (\%)} = (\text{measured concentration for fortified sample/spiked concentration}) \times 100$$

As listed in Table 2, the recoveries for three MNPs in the three control samples ranged from 64.85 to 97.51% for the

Table 1 Calibration data, LOD and LOQ, and precision of the HPLC-UV method. y: peak area; x: concentration (μg mL^{-1}); R^2: correlation coefficient

MNP	Calibration curve	R^2	Linear range (μg mL^{-1})	LOD (ng mL^{-1})	Retention time (min)	LOQ (ng mL^{-1})	Precision RSD (%) ($n = 5$)		
							Intra-day	Inter-day	Stability
2-NP	$Y = 11.646x + 0.01$	0.9990	0.001–1	0.13	13.8	0.39	1.67	1.80	4.53
3-NP	$Y = 9.4412x + 0.009$	0.9999	0.001–1	0.09	9.9	0.27	1.06	1.56	5.08
4-NP	$Y = 6.2072x + 0.006$	0.9995	0.001–1	0.19	8.3	0.57	1.14	1.62	3.99

Table 2 Recoveries of three MNPs in three kinds of fortified sample matrices ($n = 3$)

MNP	Spiking level (μg mL^{-1})	Distilled water		Tap water-1		Tap water-2	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
2-NP	0.005	64.85	10.16	56.70	1.96	59.82	3.67
	0.1	75.96	2.47	75.59	3.04	77.19	1.31
	1	94.44	3.21	89.44	2.45	90.92	3.54
3-NP	0.005	69.06	5.01	67.22	7.12	65.06	7.49
	0.1	79.21	3.50	76.25	2.04	76.34	1.45
	1	96.22	0.95	87.78	2.12	87.63	1.86
4-NP	0.005	69.29	6.15	66.16	6.16	68.86	2.42
	0.1	82.99	2.43	77.76	0.99	74.37	0.91
	1	97.51	1.33	95.16	1.24	96.28	6.94

distilled water sample, 56.70–95.16% for Tap water-1, and 59.82–96.28% for Tap water-2, with RSD values in the range of 0.91–10.16%. These results demonstrated that the developed method was precise, accurate, and sensitive enough for simultaneous determination of trace levels of three isomeric MNPs in water samples.

4. Results and discussion

4.1 Characterisation

Fig. 1C and ESI S1A, B† show the FT-Raman and FT-IR spectra of chitin and *p*-nitrophenol loaded chitin powders. As can be seen, no new band appears or disappears in the full range FT-IR or FT-Raman spectra of the *p*-nitrophenol loaded sample compared to the chitin one. This probably means that the interaction of chitin with the nitrophenol is given by weak interactions. On the other hand, in the FT-Raman spectra (Fig. 1C), it could be seen that the amide I band presents a shift from 1657.5 cm^{-1} to 1653.7 cm^{-1} when PNP interacts with chitin. This band is known to be conformation sensitive, presenting specific positions for α-chitin and β-chitin spectra, which present different interchain hydrogen bondings.[28,29] Therefore, it could be proposed that the shift observed in the amide I band of the PNP loaded chitin spectrum accounts for a restricted conformation driven by a hydrogen bonding between the –OH of the nitrophenol and the C═O or –N–H of the acetoamide group of chitin. In addition, this effect could be due to other interactions that could alter the interchain hydrogen bonding of chitin. Thus, this could not be considered the only H bonding possible between both compounds.

4.2 Optimization of chitin based solid phase extraction

Several parameters that influenced the performance of chitin based SPE, such as the type and amount of sorbent, washing solvent, eluting solvent, sample volume, pH, ionic strength and flow rate, were investigated in order to obtain the highest possible high recovery rates of the target MNPs. All the graphs are shown as mean ± standard deviation (S.D.).

4.2.1 Sorbent selection. Because environmental waters are complex matrices, one of the most important steps in the optimization of the SPE procedure was to select an appropriate sorbent that could effectively enrich the analytes. Two sorbents were tested and compared, including C_{18} and chitin. After comparison, chitin was chosen as a sorbent due to its low cost, easy cleanup, and high recoveries as compared to the C_{18} (ESI Fig. S2A†).

4.2.2 Sorbent amount. As chitin was used as a sorbent, the amount of sorbent needed to effectively remove target MNPs was determined. A large amount of sorbent can effectively purify MNPs in water matrices, but with low overall recoveries. To determine the optimum sorbent level for purification efficiency, different amounts of chitin (10, 50, 80, 100, 150 mg) were tested and compared. Results showed that using 100 mg chitin provided satisfactory purification efficiency as well as high recoveries for all three target MNPs in water samples (ESI Fig. S2B†).

4.2.3 Sample volume. Volume of the loaded sample has a proportional effect on enrichment factor. Therefore, various volumes (1–6 mL) of the samples were tested. The results are shown in Fig. 2A. Considering the need for high recovery and

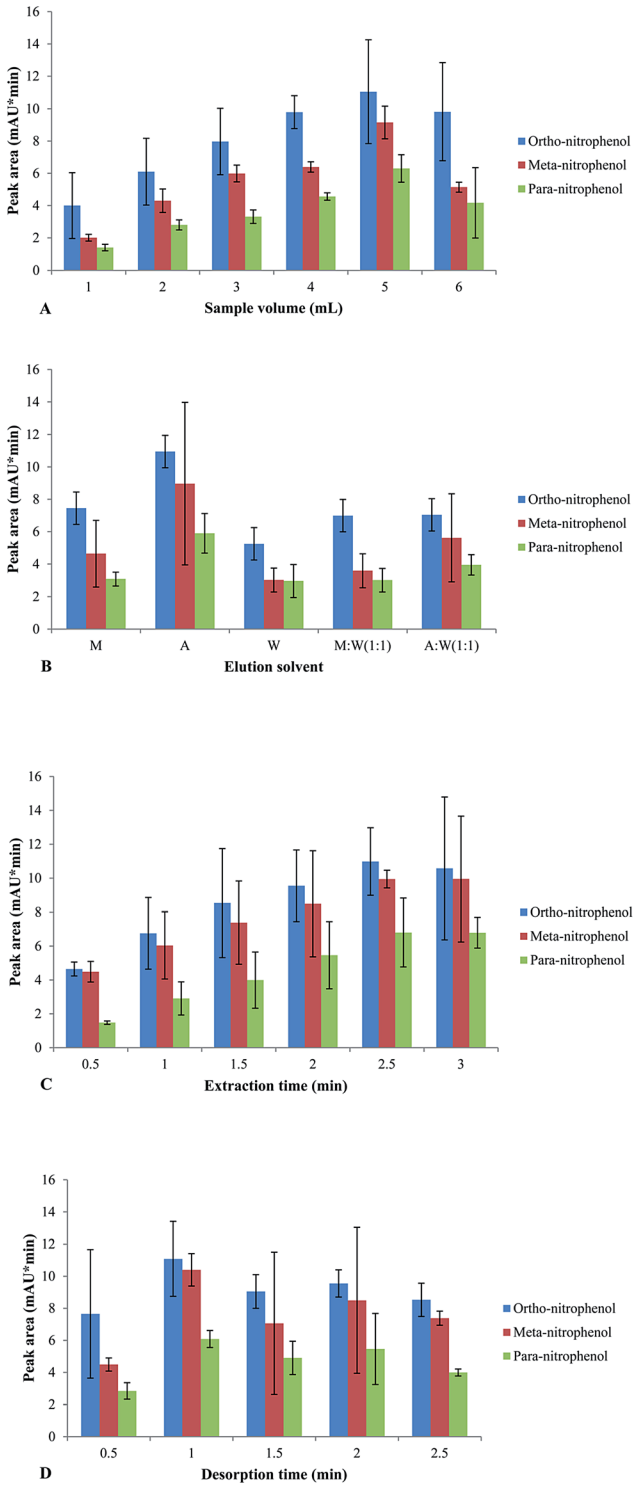

Fig. 2 (A) Effect of sample volume in the SPE procedure. (B) Effect of elution solvent/solution in the SPE procedure. (C) Effect of extraction time in the SPE procedure. (D) Effect of desorption time in the SPE procedure.

high enrichment factor, we selected 5 mL as an appropriate water sample volume above which the recovery of all three MNPs decreased.

4.2.4 Effects of sample pH and ionic strength. The effect of sample pH ranging from 3.0 to 11.0 on the adsorption of chitin is shown in ESI Fig. S2D.† The amount of three MNPs adsorbed on chitin increases with increasing initial pH up to 7 and then decreases with further increase in pH. The effect of pH on MNP adsorption by chitin mainly results from the distribution of two MNP species, the molecular and the anionic. All three MNPs are water-soluble solids and are weak acids in water (2-NP pK_a = 7.2, 3-NP pK_a = 8.3 and 4-NP pK_a = 7.1). The molecular MNPs can be effectively adsorbed onto chitin at pH 7 by hydrogen bonds. However, when the pH value is below or equal to 6, damage occurs to the chitin crystalline structure, leading to the weak binding force for MNP adsorption.[20] Moreover, taking into account that chitin presents a certain amount of amino groups, below pH 6, it could be considered the contribution of an ion–exchange interaction of this group and the deprotonated species of the phenol groups. The MNP exists as a phenolate anion when the pH is higher, and this group would not be available to form H bonds with chitin. In addition, the solubility of these compounds would be higher, decreasing the probability of interaction.

In the present study, the effect of ionic strength in the matrix was investigated by addition of NaCl from 0 to 25% (w/v) (ESI Fig. S2E†). The results showed that the extraction performance of chitin for MNPs increased when suitable NaCl was added. Under the salt-out effect, 20% NaCl showed the maximum extraction efficiency for all MNPs and thus, the addition of 20% NaCl was used in the following study.

4.2.5 Elution solvent and its volume. As far as the SPE method is concerned, MNP desorption from the chitin can significantly affect the sensitivity of the MNP extraction. Thus, proper elution of the solvent plays a key role in the process. In the present study, methanol, acetonitrile, water and their mixtures were selected as possible solvents in this experiment. Results indicated that the extraction performance reached a maximum for all studied MNPs when acetonitrile was chosen (Fig. 2B). The volume of the elution solvent was also studied, and the efficient elution could only be achieved with 250 μL of acetonitrile; moreover, increasing the volume of eluent reduced the enrichment factor (ESI Fig. 2C†). To ensure complete elution, the essential and sequential elution with another 250 μL of acetonitrile revealed the absence of MNPs in the chitin.

4.2.6 Washing solvent. Selection of a suitable washing solvent is essential for the development of a useful SPE method. In order to achieve high extraction efficiency, organic solvents with different polarities and levels of water solubility were tested. Commonly used solvents with a wide range of polarities, including acetonitrile, methanol and water, were tested.

Table 3 MNPs in 8 real water samples[a]

Sample	MNP detected	Concentration (ng mL^{-1})
Agricultural run-off-1	4-NP	913.09
Agricultural run-off-2	4-NP	832.76
Industrial waste water-1	4-NP, 2-NP	947.52, 480.64
Industrial waste water-2	4-NP, 2-NP	749.16, 278.09
River water-1	n.d.	—
River water-1	n.d.	—
Tap water-1	n.d.	—
Tap water-2	n.d.	—

[a] n.d.: not detected.

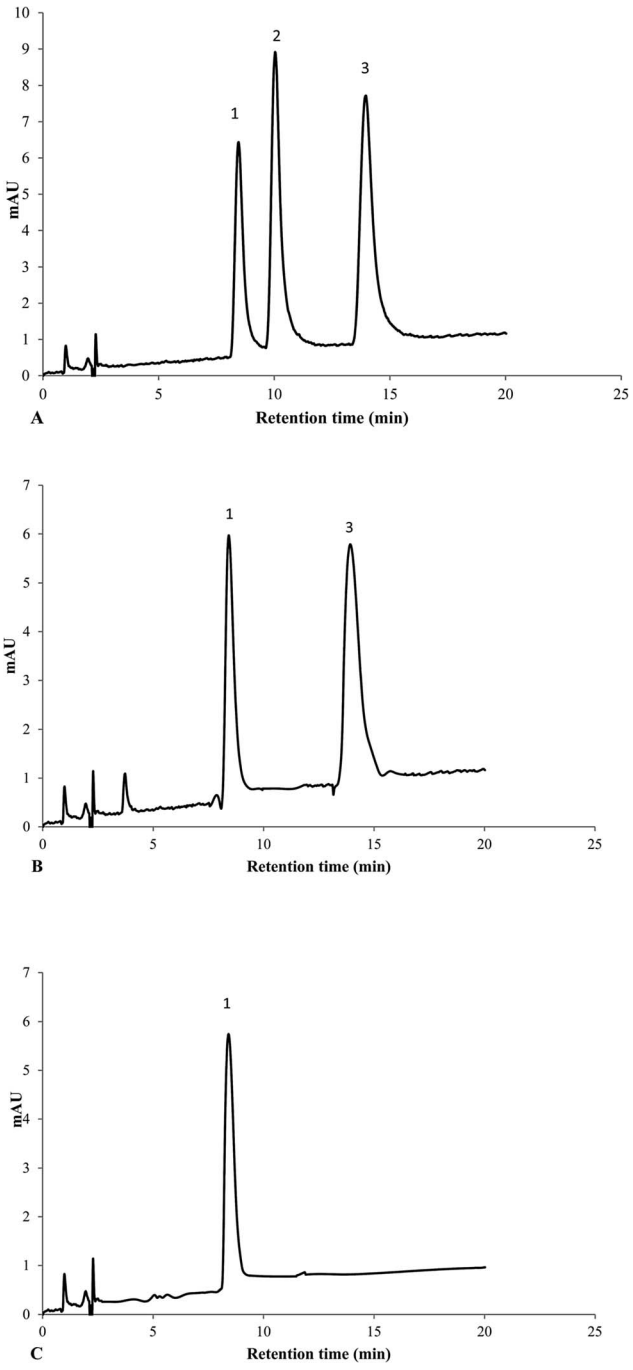

Fig. 3 (A) SPE-HPLC-UV chromatogram of fortified distilled water sample with three MNP standards (0.4 μg mL^{-1}). Peaks: 1,4-NP; 2,3-NP; 3,2-NP. (B) SPE-HPLC-UV chromatogram of industrial waste water-1. (C) SPE-HPLC-UV chromatogram of agricultural runoff-1.

Table 4 Performance comparison between current work and the existing methods[a]

Analytes	Method	Linear range (μg mL^{-1})	LOD (ng mL^{-1})	RSD (%)	Ref.
2-NP	SPME-GC-FID	3.0–20 000	0.9	8.4	30
4-NP	IP-SAME-HPLC-DAD	0.2–75	0.1	3.4	31
2-NP, 4-NP	SPME-HPLC-UV	5–30 000	1.6–4.1	≤11.3	32
4-NP, 2,4-DNP	MS-USAEME-SFO-UHPLC/PDA	2.5–1000	0.6–3.2	6–10	33
2-NP, 4-NP, 2,4-DNP	USAEM-HPLC-UV	1–200	0.25–1	<4.2	34
2-NP, 4-NP, 2,4-DNP	SPE-HPLC-UV	0.2–200	—	0.1–10.3	35
2-NP, 4-NP, 2,4-DNP	MSPE-HPLC-UV	0.75–100	0.3–0.4	2.8–4.9	36
2-NP, 3-NP, 4-NP	SPE-HPLC-UV	0.001–1	0.09–0.19	1.06–1.80	Current work

[a] IP-SAME: ion pair based surfactant-assisted microextraction. SPME: solid-phase microextraction. MS-USAEME-SFO: manual shaking-enhanced, ultrasound-assisted emulsification microextraction method based on solidification of a floating organic droplet. USAEM: ultrasound-assisted emulsification microextraction. SPE: solid-phase extraction.

Specific mixtures that were tested included methanol with water (1 : 1, v/v) and acetonitrile with water (1 : 1, v/v). Using methanol, acetonitrile, and their (1 : 1 v/v) mixtures as washing solvents, precise quantification was difficult because the fortified samples were not satisfactorily purified, and interfering peaks were observed simultaneously at the retention times of target MNPs; pure water gave higher average peak areas at the retention times of target MNPs and was therefore the preferred washing solvent (ESI Fig. S2F†).

4.2.7 Extraction time and desorption time. In order to further assess the ability of chitin to extract MNPs, the extraction time profiles were investigated by increasing the extracting time from 0.5 min to 3 min. As shown in Fig. 2C, the extraction performance increased sharply when the extraction time increased from 0.5 to 2.0 min. The good slopes of the profiles indicated that the chitin possessed a good extraction capacity towards three MNPs. At the same time, the extraction equilibrium was reached after 2.5 min. Consequently, the extraction time of 2.5 min was selected for further studies. Desorption time was investigated ranging from 0.5 min to 2.5 min when the extraction time was kept at 2.5 min. The results showed that the MNPs could be eluted from the chitin completely in 1.5 min when the extraction time was 2.5 min (ESI Fig. S2G†).

4.2.8 Recyclability of chitin. The regenerability of chitin was examined using methanol and acetonitrile. The MNPs' adsorption performance of chitin regenerated by acetonitrile is superior to that renewed by methanol. After five adsorption/desorption cycles using acetonitrile, the MNP uptake amount still remains equivalent, indicating that the acetonitrile is very suitable for the regeneration of acetontrile, and chitin could be a cost-effective and promising adsorbent for MNP enrichment.

5. Real sample analysis

The validated chitin based solid phase extraction procedure coupled with HPLC-UV protocol was applied to determine three target MNPs in 8 water samples collected from various sources (Section 2.5). As listed in Table 3, two MNPs, namely 2-NP and 4-NP, with contents above the LOQ were detected in 4 samples, whereas the other 4 samples tested negative for the target MNPs. Examples of the positive samples included two agricultural run off and two industrial waste water samples. Respective, representative chromatograms are shown in Fig. 3B and C and ESI S3B, C.† Out of the detected MNPs, 4-NP was the most predominant, with concentration levels ranging from 749.16 to 947.52 ng mL^{-1} in the four samples, followed by 2-NP, ranging from 278.09 to 480.64 ng mL^{-1} in two samples.

6. Comparison with other methods

The present SPE-HPLC-UV method with the chitin as adsorbent was also compared with other methods reported in literature (as shown in Table 4).[30–36] The concentration linear range, LODs, and RSD were compared. The LODs achieved in the present research are lower or comparable with those reported in the literature. Furthermore, chitin as an adsorbent has a major advantage that no laborious synthesis is needed and is a cheap, green, and biocompatible material. Therefore, chitin based SPE-HPLC-UV method is proven to be a green, convenient, efficient and reliable method for the enrichment of trace MNPs from environmental water samples.

7. Conclusion

Simultaneous analysis of three isomeric mononitrophenols is a topic of interest in the field of analytical chemistry. Preparation of MNP enriched samples is often the main constraint for successful analysis. In this study, a simple and rapid method combining a chitin based solid phase extraction procedure with HPLC-UV was developed and validated for simultaneous monitoring and identifying trace levels of three isomeric MNPs in water samples. Crucial parameters for SPE extraction and chromatographic analysis were optimized, and the developed method was validated. The final method provided a wide concentration range, satisfactory linearity, low LOD and LOQ, good precision, and a high recovery rate, which was comparable with other detection methods for trace levels of nitrophenols. Next, the method was used to simultaneously analyze three MNPs in 8 water samples. This successful real world application demonstrated that though the validated method used

economical, green, cheap, and simplified extraction and clean-up procedures, it still maintained adequate sensitivity for detection of trace levels of MNPs and could easily be adopted by other laboratories for the analysis of complex matrices and could be used as a powerful reference for trace analysis of other NPs or other nitrogen-containing pollutants.

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