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ARTICLE

Dual application of synthesized SnO_2 nanoparticles in ion chromatography for sensitive fluorescence determination of ketoprofen in human serum, urine and canal water samples

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Abstract

The aim of this novel study is the introduction of a cheap, simple, sensitive and green methodology involving the dual application of the synthesized porous SnO_2 nanoparticles (NPs) for selective conversion of non-fluorescent ketoprofen (KP) into a highly florescent specie and as a sorbent in a µ-sample preparation method used for extraction of KP from three complex human serum, urine and canal water samples. The clean separation and sensitive fluorescence determination of KP from these complex samples was carried out by coupling an ion chromatograph with a fluorescence detector (IC-FLD). The sorbent prepared by a simple chemical precipitation method in water and was characterized by various techniques. The porous SnO_2 NPs along with selective conversion of KP into a highly fluorescent specie also proved an effective sorbent for the selective degradation and elimination of polar organic, inorganic matrices and heavy metals in complex samples. The optimized analytical method exhibited satisfactory linearity for ketoprofen in a concentration range of 0.2 – 1.5 mg/kg with a correlation coefficient (r^2) of 0.997. The limit of detection (LOD) and quantification (LOQ) in human serum, urine and canal water samples were 0.1 µg/kg, 0.5 µg/kg, 0.39 µg/kg and 1.3 µg/kg 0.3 µg/kg, 1.7 µg/kg, respectively. The method also showed good intra-day and inter-day precisions at two concentration level 0.5 mg/kg and 1.3 mg/kg in complex samples with relative standard deviations (RSDs) less than 16.3% (n=5) and satisfactory recoveries were retrieved in the range of 85.1–101.4% with minimum or no matrix effect.

Keywords: Ketoprofen; SnO_2 nanoparticles; µ-sample preparation; Ion chromatography; Fluorescence detection

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1. Introduction

The non-steroidal anti-inflammatory drug (NSAID), ketoprofen (KP) usually prescribed for the medication of osteoarthritis, rheumatoid arthritis, gout, ankylosing spondylitis and subduing pain as a consequence of vascular headaches, dysmenorrheal, rheumatic and non-rheumatic inflammatory disorders. However, ketoprofen poor solubility in water, its ability to adsorb, metabolize and excrete in no time has sprang out various renal and gastrointestinal effects like as dyspepsia, nausea, diarrhea and constipation. In addition, various NASIDs along with KP are directly indirectly contaminating every environment compartment and enhancing the bacterial resistance against these drugs, as a result, ketoprofen and other pharmaceuticals drugs now have been recognized as 'emerging' contaminants [1-3].

In order to its trace determination, various chromatographic and non-chromatographic techniques have been used for this purpose after separately extracting it from various samples by using different rigorous, expensive, huge organic solvent consuming and laborious extraction methods including solid-phase extraction (SPE) [4-6], liquid–liquid extraction (LLE), hollow fiber-based liquid phase microextraction (HF-LPME) [7] and QuEChERS method [8].

However, previously used analytical techniques like time resolved luminescence [3], gas chromatography-mass spectrometry (GC-MS) [9], micellar liquid chromatography and HPLC coupled with ultraviolet (UV) detector [10, 11], liquid chromatography coupled with mass spectrometry (MS) [12], HPLC separation and UV induce [13, 14] and chemical derivatized fluorescence detection [15] have serious limitations. For example, HPLC-ESI-MS technique is suffered by analyte signal suppression or enhancement effect during its electrospray ionization in complex samples analysis. Beside, these expensive and heavy instrumentations are still not available in every research institute and laboratories [14]. Whereas, photometric UV and fluorescence determination methods are commonly used detection techniques; but former lack sensitivity and selectivity, while later requires chemical and photochemical derivatization for its detection. However, despite using complex system poor detection limit of 1.45 mg L^{-1} was obtained. In addition, under UV irradiation KP can also decompose into nine other hazardous photoproducts. These free radical intermediates further can react with biological macromolecules (e.g. DNA, fatty acids, lipids, membranes) and result in toxicity of the drugs. This method have also affected the fluorescence quantum yield of other analytes [13, 14, 16].

Therefore, to overcome the limitations of these sample preparation and to reinstate the sensitive and selective fluorescence detection technique for KP determination in various complex samples, the porous SnO_2 NPs were synthesized by simple chemical precipitation method by following A. Bhattacharjee *et al.* scheme [17] with little modification. These synthesized porous SnO_2 NPs were used as sorbent in a new micro sample preparation method to remove polar

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matrices/interferences (fatty acids, lipids, fats, pigments, carotenoids), carcinogenic heavy metals, pigments, dyes under sunlight and for selective conversion of non-fluorescent KP into a strong fluorescent specie, which sensitively determined by IC-FLD. Since last decade, ion chromatography proved to be a better alternative to HPLC due to its column compatibility with all solvents and stability in wide pH range 1-14 [18].

Recently, a number of authors have explored the application of different NPs and nanotubes as a nanosized SPE adsorbents for matrix elimination and enrichment of analytes of interest. Most probably, the growing interest in nanomaterials as sorbents over SPE sorbents is due to their: (a) high surface areas; (b) diffusion route; (c) very high extraction capacity; (d) quick extraction dynamics; (e) high extraction efficiencies; (f) do not agglomerate during sample preparation; (g) easily separated from matrix by centrifugation or by applying a magnetic field; (h) organic contaminant or others can easily desorbed by the change of pH and (i) potentially NPs can be recycled [19-26].

Therefore, SnO_2 NPs were synthesizes by simple chemical precipitation method and their size and morphology were controlled by using glycine and sodium dodecyl sulphate (SDS) surfactant as capping/complexing and stabilizing agent, respectively [17].

This manuscript for the first time is introducing the dual role of porous SnO_2 NPs in ion chromatography for selective conversion of non-fluorescent KP into highly fluorescent specie for its sensitive determination in complex samples and as a green sorbent in micro sample preparation method used for the matrices/interferences elimination (polar interferences, dyes, heavy metals) in human serum, urine and canal water samples.

2. Experimental

2.1. Instrumentation

The IC-FLD separations and analyses were performed using a Thermo Fisher Scientific Ultimate ICS-1500 system (Waltham, MA, USA) equipped with an isocratic dual-piston pump with a serial number 08030677, a six-port valve with two ports fitted with a 25 µL sampling loop and a column heater. Further, separation was carried out using IonPac® AS12A column (250 mm × 4 mm i.d; 13 mm particle size) safeguarded by an IonPac® AG12A guard column (50 mm × 4 mm i.d; 13 mm particle size) and it is connected with model Ultimate 3000 RS fluorescence detector (Dionex, Sunny vale, CA, USA). The electronic signal were collected and analyzed with Chromeleon™ 7.2 software (Thermo Fisher Scientific, USA). The SB-5200DT ultrasonic cleaner (Scientz Biotechnology Co. Ltd., Ningbo, China) was used for sonication of samples. The NPs characterization was carried out by powder X-ray diffraction (XRD) method using Phillips X'Pert PRO diffractometer. The scanning electron microscopy (SEM) and transmission electron microscopy (TEM) images were attained using

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Hitachi SU-70 (Hitachi Japan) and Hitachi Model H-7650 transmission electron microscope (Hitachi Japan), respectively. Fourier transform infrared (FTIR) spectroscopy was carried out (SGE / Agilent 6890 / Nicolet 5700) spectrometer (USA).

2.2. Chemicals and materials

Analytical standardof ketoprofen (KP), (≥98%, w/w) was obtained from Aladdin industrial Co. Ltd. (Shanghai, China). The other reagents used for SnO_2 NPs, sample and eluent preparation were sodium hydroxide, extra pure (50%, w/v solution in water), sodium chloride (≥99.5%, w/w), magnesium sulphate (99.0%, w/w), glycine (99.5%, w/w), sodium dodecyl sulfate (SDS), (≥85%, w/w), sodium hydroxide (98.9%), sodium chloride (99.9%) and tin (II) chloride dihydrate (≥98.5%, w/w), which were obtained from Huipu Co. (Hangzhou, China) and Aladdin industrial Co. Ltd. (Shanghai, China). Real canal water sample was collected from a canal passing near to Zhejiang University hospital in amber glass bottle pre-cleaned with methanol. The other real human serum and urine samples were collected from the healthy volunteer in the Zhejiang University hospital. All sample bottles and vials covered with aluminum foil and were stored in the refrigerator at 4 $^{\circ}$C. The informed consent of volunteers was obtained regarding experimentation. All experiments were performed in compliance with the Hangzhou government and Zhejiang university guidelines, and department of chemistry of Zhejiang University have approved the experiments.

2.3. Preparation of SnO_2 NP

Previously reported chemical precipitation method with minor modification was used for the synthesis of SnO_2 NPs [17]. Briefly, 0.02 M $SnCl_2.2H_2O$ and 0.02 M glycine aqueous solution were separately prepared in 100 ml measuring flasks. These two solutions were poured into a 500 ml beaker. Another 40 ml aqueous solution of 60 mmole sodium dodecyl sulphate (SDS) surfactant was prepared and filled in a 100 ml burette. This solution was added dropwise in 500 ml beaker containing the mixture with continuous stirring at room temperature. Later, the reaction mixture was put on oil bath and stirred at 100 ^{o}C for 3-4 hour, until mixture turned yellow. These yellow precipitates were left overnight in dark at room temperature. Next day, the precipitates were centrifuged and washed twice with deionized water. Finally, precipitates were dried at 60 ^{o}C and then calcined at 600 $^{\circ}$C for two hours. The synthesized SnO_2 NPs were characterized and stored in a dry bottle for subsequent use in analytical experiments. The schematic layout of SnO_2 NPs synthesis and their dual application in ion chromatography is shown in Fig. 1.

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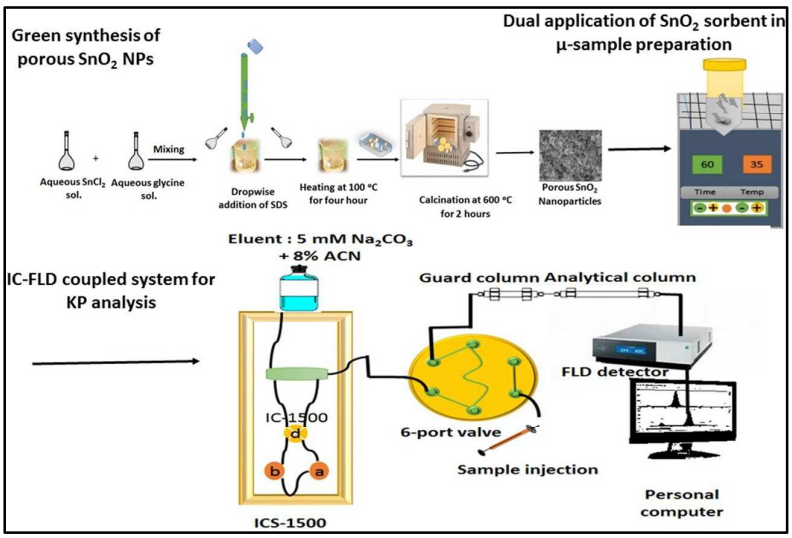

Fig. 1 Systematic display of dual application of synthesized SnO_2 NPs in ion chromatography for the selective determination of non-fluorescent KP in three complex samples.

2.4. µ-sample extraction method

The simple µ-sample extraction method involved following steps:

Step 1: The powder form 60 µg SnO_2 sorbent precisely weighed and conditioned with 300 µL water in 4.0 mL centrifuge vial and was put on sonication bath, meanwhile, 300 µL of each $NaBH_4$ and respective sample were mixed and spiked with 0.5 or 1.3 mg/kg KP in another 4.0 mL centrifuge tube and the resulting mixture was vigorously shaken for few seconds with hand.

Step 2: The mixture of second centrifuge tube was poured into a centrifuge tube containing the sorbent and it was put on sonication bath for 60 minutes at 40 °C in order to convert non-fluorescent KP into fluorescent specie and for degradation and adsorption of organic and polar matrices and heavy metals onto the sorbent.

Step 3: After 60 minutes the centrifuge tube was centrifuged at 9500 RPM for 5 minutes to stop reaction. After centrifugation 70 µg NaCl + 140 µg $MgSO_4$ along with 900 µL ACN were added and sonicated for another 12 minutes on sonication bath to have maximum analyte back extraction in ACN and to disperse impurities and matrices in aqueous phase.

Step 4: Finally, centrifuge tube was centrifuged at 9500 RPM for 12 minutes to settle down the sorbent. After centrifugation two layers appeared, upper layer was decanted into another vial. The resulting supernatant was dried under the gentle stream of inert nitrogen gas. The dried residues

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were reconstituted to 900 µL with acetonitrile: water (50%, w/w) mixture and injected in the IC-FLD system through 0.22 µm membrane filter for analysis.

The same procedure was adopted for other samples, except human serum was first dissolved in 1/3 acetonitrile in order to precipitate the protein and to homogenize the serum.

3. Result and Discussion

3.1. Characterization of synthesized SnO_2 NPs

XRD measurement was conducted to investigate the overall phase composition and structure of the synthesized SnO_2 NPs. Fig. 2(a) shows peaks appeared at 2θ = 26.8 °, 34.13 °, 39.28 °, 52.05 °, 55.04 °, 58.13°, 62.23°, 65.01°, and 66.18° which corresponds to the lattice plane (110), (101), (200), (211), (220), (002), (310), (112), and (301), respectively. The appeared peaks positions completely match with the reported tetragonal rutile structure of SnO_2 nanoparticles. This endorsed the previous studies that have confirmed the tetragonal rutile structure of SnO_2 NPs [17]. The IR spectra of the powder SnO_2 NPs was also measured in the wave number range of 400 to 4000 cm^{-1} using FT-IR spectroscopy. The characteristics peak appeared at 660–610 cm^{-1} are attributed due to Sn-O-Sn stretching mode of surface bridging tin oxide nanoparticles and the bands obtained around 530-560 cm^{-1} are designated to the terminal Sn-O vibration of Sn-OH group as shown in Fig. 2(b). Few small peaks were also appeared around 3433 cm^{-1} that confirm the existence of –OH groups. The high intensity peak around 618 cm^{-1} is indication of maximum conversion of Sn–OH into Sn–O–Sn at 600 °C [17, 27].

$$\mathrm{Sn-OH} + \mathrm{Sn-OH} \rightarrow \mathrm{Sn-O-Sn} + \mathrm{H-O-H}$$

The synthesized SnO_2 NPs size distribution and morphology is shown by SEM and TEM images given in Fig. 2(c-f). The SEM and TEM images depicts the monodisperse nature of the SnO_2 NPs with average size is about 10-13 nm. The average diameter of fifty random NPs was calculated with the help of software (Nano Measurer 1.2.5 20150222) and ~11.92 nm was found with RSD of 2.09% as given in Fig. S1. The lattice spacing is calculated from the TEM image and found to be 0.174 and 0.141 nm which corresponds to (211) and (301) lattice plane. From the TEM images it is also evident that the SnO_2 NPs are spherical and polycrystalline in nature with an average particle size of 10-13 nm, respectively, and these nanospheres are made up of individual nanocrystallites. The overlapping spherical NP's are reflecting the porosity and rough surface of NP's, and these spherical surface enhanced their electron conducting and matrices adsorption capability [28, 29].

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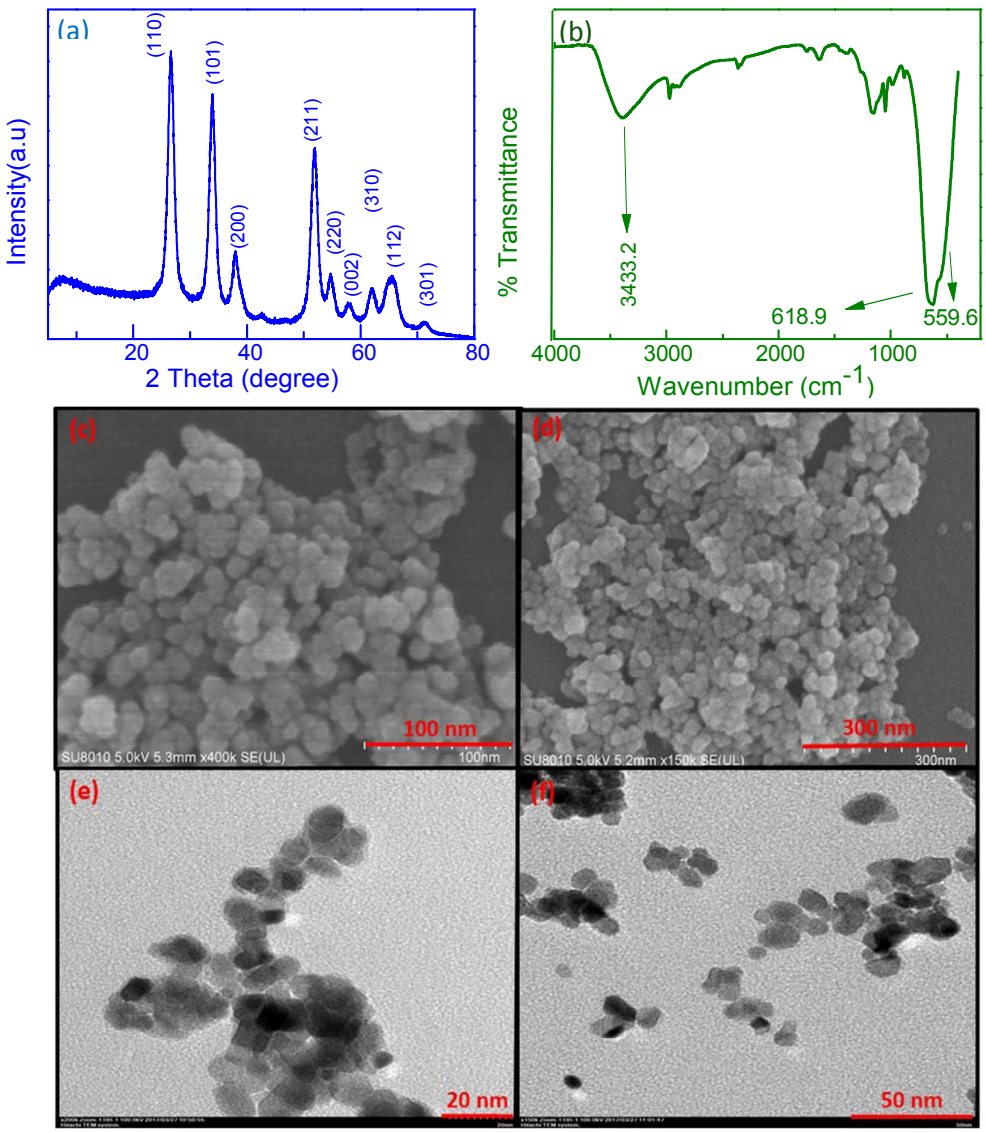

Fig. 2. (a) XRD pattern of synthesized SnO_2 nanoparticles; (b) FT-IR spectra of SnO_2 nanoparticles and (c-f) SEM and TEM images at different resolution.

3.2. Scanning and ion chromatographic separation condition of KP

The reduced KP was scanned with model Ultimate 3000 RS fluorescence detector (Dionex, Sunny vale, CA, USA) in basic media and $\lambda_{em}/\lambda_{ex}$ = 229 / 292 nm were appeared as optimum wavelength for its sensitive fluorescence detection as shown in Fig. S2.

An isocratic separation of KP in the presence of other four most commonly used pharmaceutical drugs was carried out by using an anion-exchange IonPac® AS12A column connected with IonPac®

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AG12A guard column. This column isocratically separate all five available acidic drugs with sodium carbonate and acetonitrile as eluent. All drugs were finely separated with good peak shape by using 5 mM sodium carbonate and 10% acetonitrile as a eluent; at this condition all kind of ion–ion (anion-exchange) interaction, π–π interaction and dipole–dipole interaction with the cationic alkyl quaternary ammonium ion functional groups and polystyrene-divinely benzene (PS-DVB) copolymer of the column stationary phase were slowly overcame by this eluent. As a result, ciprofloxacin (CIP), clofibrate (CLO), ibuprofen (IBU), ketoprofen (KP), and flurbiprofen (FLU) were eluted sequentially based on their anion-exchange interaction strength. This elution order was mainly controlled by ion–ion (anion exchange) interaction rather than hydrophobic interaction. The higher retention time of FLU can be attribute to the strong force of attraction between cationic alkyl quaternary ammonium ion groups of column bed and highly charge density fluoro and ionized carboxyl group, while the π–π interaction of the planer structure of KP with column bed helped it to adsorb for a little longer time. These strong forces caused peak width and peak tailing, which were controlled by addition of 10% acetonitrile in eluent. This small addition of ACN in eluent not only has avoided peak broadening and tailing but also gave better peak shape, resolution and also have improved the fluorescence intensity of all five acidic drugs in basic media. The clean separation is given in Fig 3.

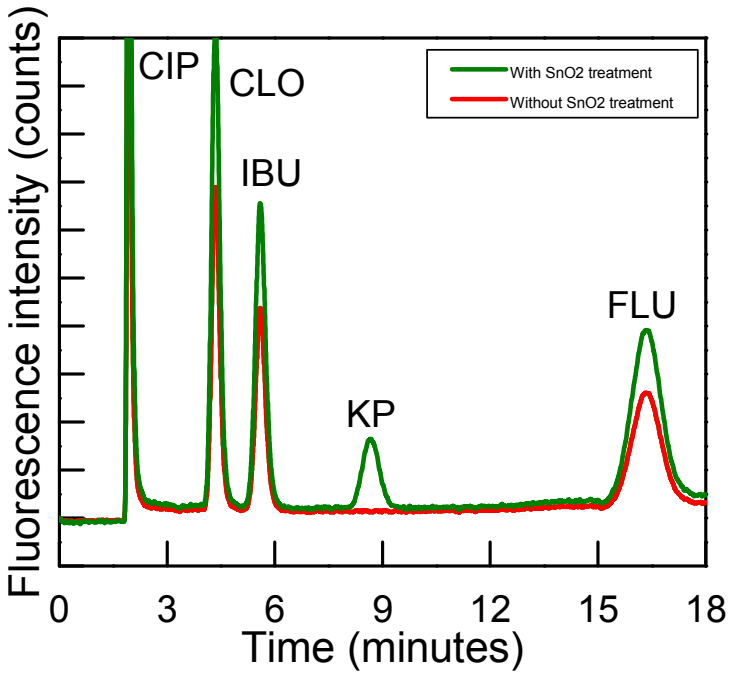

Fig. 3. Chromatogram of isocratic separation of 1 mg/kg CIP, CLO, IBU and KP and and 10 µg/kg FLU standard. Mobile phase: 5 mM Na_2CO3 + 10% ACN; columns: IonPac® AS12A column (250 mm × 4 mm i.d; 13 mm particle size) preceded by an IonPac® AG12A guard column (50 mm × 4 mm i.d; 13 mm particle size); injection volume: 25 µL; temperature: ambient; flow rate: 1.0 mL/min; FLD

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detector wavelength: λ_{ex} / λ_{em} for CIP = 295/340 nm, λ_{ex} / λ_{em} for CLO and IBU= 229/329 nm, λ_{ex} / λ_{em} for KP = 229/292 nm and λ_{ex} / λ_{em} for FLU = 235/315 nm.

The same mobile phase 5 mM Na_2CO_3 and 10 % acetonitrile (ACN) at flow rate of 1.0 mL/min was also chosen for its clean and rapid separation of alone KP in pure acetonitrile and complex samples. The clean separation of 0.5 µg/ml standard KP with and without use of SnO_2 NPs sorbent is shown in Fig. 4(b).

Contrary to other four pharmaceutical drugs, KP normally cannot be directly detect with fluorescence detection techniques; however after SnO_2 sorbent treatment it turned into a highly fluorescent specie as it is elaborated in reaction scheme in Fig. 4. The fluorescence property of reduced KP can be linked to increment of delocalization path up to two benzene rings of KP in the presence of electron donating functionalities. This effect was more pronounced after hydroxyl and acidic protons ionization in basic media. These ionized forms also have diminished the H-bonding effect with solvent, consequently, the internal conversion is reduced which results in high fluorescence intensity [30].

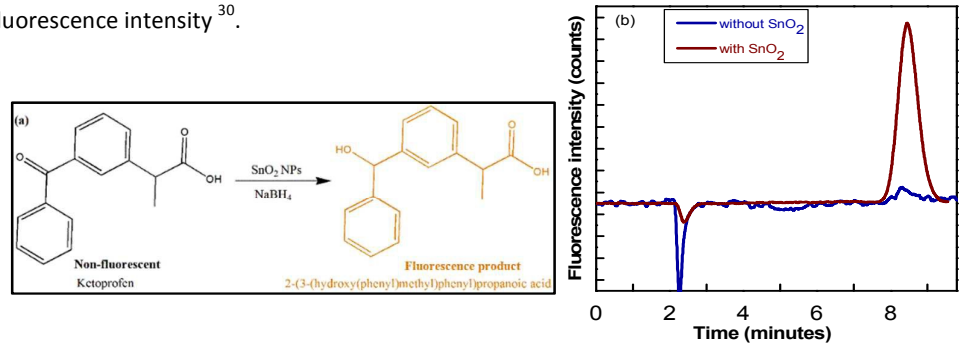

Fig. 4 (a) Chemical conversion of KP into fluorescent product and (b) fluorescence chromatograms at $\lambda_{ex}/\lambda_{em}$ = 229 / 292 nm of KP 0.5 µg/kg standard solution without SnO_2 treatment (blue line) and with SnO_2 treatment (wine red line).

The confirmation of reduced fluorescent ketoprofen (R-KP) specie structure was carried out by MS study. The standard solution of R-KP was collected from IC-FLD system effluent. This collected sample was concentrated under gentle stream of nitrogen and later directly infused into the LC-MS system. LC-ESI-MS/MS of R-KP was investigated systematically to choose precursor ions by comparing the four ways of $[M+Na]^+$, $[M+NH_3]^+$, $[M+H]^+$ and $[M-H]^-$. Finally, we chose $[M+H]^+$ as the precursor ion because this ion was the most abundant peak in the mass spectra, and MS/MS spectra of the precursor ion at m/z 257.3 is shown in Fig. 5. The MS/MS spectrum was obtained m/z 76.7 and m/z 105.0, corresponding to the fragmentation ions of $[M + H - C_4H_7O_3]^+$ and $[M + H - C_8H_8O_3]^+$ from the m/z precursor ion (m/z 257.3), respectively. The 257.3→76.7 and 257.3→105.0 transitions were selected as the quantification and identification transitions, and the cracking pathway are also shown in Fig. 5.

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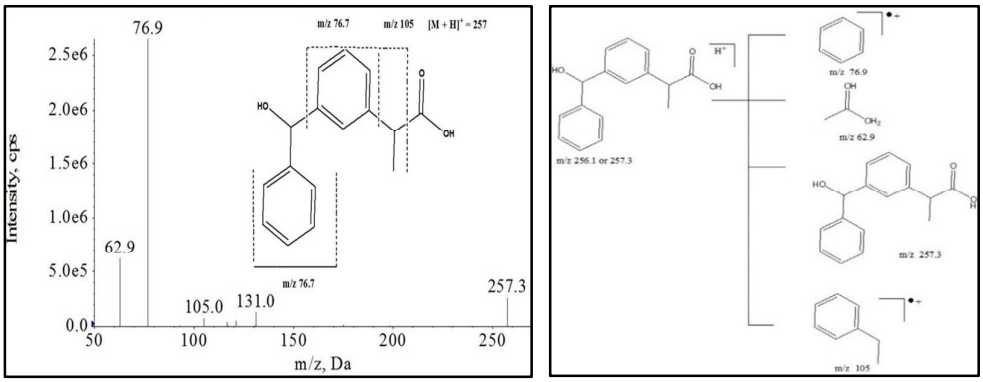

Fig. 5 Mass spectrum of the reduced fluorescent KP with fragmentor potentials set at 100 V

3.3. Optimization of KP fluorescent intensity affecting parameters

There were few parameters which directly or indirectly have affected NPs activity and fluorescence intensity of reduced KP, it is extremely important to optimize these parameters in order to have optimum fluoresce intensity and recovery of KP from complex samples. These few parameters are evaluation of different capping agents on SnO_2 sorbent efficiency, evaluation of calcination temperature, optimization of $NaBH_4$ concentration, sorbent amount and optimization of reaction time. Each and every experiment was performed in quintet and their relative standard deviations (RSDs) were also calculated (n = 5).

3.3.1 Evaluation of different capping agents and calcination temperature on SnO_2 sorbent efficiency

The three different capping agents namely ammonia, glycine and urea used for SnO_2 sorbent synthesis and their effects on KP fluorescence quantum yield was observed. The capping agents normally used to influence NPs morphology, size and properties [31]. However, NPs synthesized using glycine and SDS relatively have enhanced the KP fluorescence intensity as compare to other two capping agents, it can be observed from Fig 6a. This may be due to the much smaller size of NPs in case of glycine; as small size NPs have large surface area and high catalytic activity.

Calcination temperature have also impart main role in final shaping of synthesized NPs. It was observed the KP fluorescence intensity and peak shape were much improved when calcination temperature was 600 °C; whereas, the distorted peak shape was appeared at 200 °C as shown in Fig. 6c. This can be attributed with inability of low calcination temperature to complete elimination of surfactant and stabilizing agents from NPs surface, which entailed incomplete reduction of KP into fluorescent specie. Whereas, high calcination temperature helped in dissolution of the nanospheres, complete elimination of surfactants, additives and impurities which results in more porous and high surface area of porous SnO_2 NPs [17, 29].

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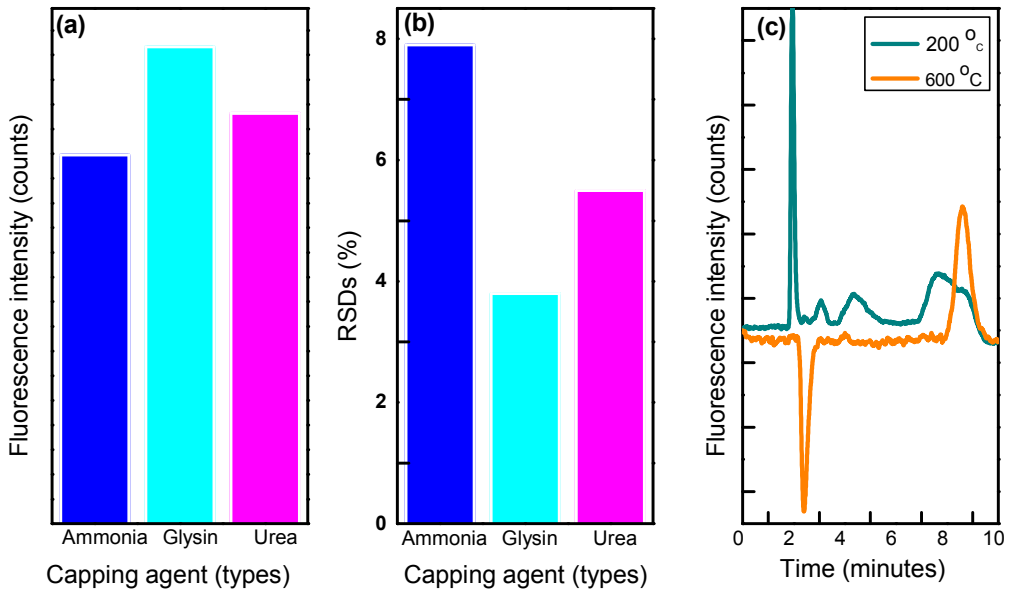

Fig. 6 (a) Effect of different capping agents on KP fluorescent intensity; (b) RSDs of each capping agent (n = 5) and (d) effect of calcination temperature on KP fluorescence intensity.

3.3.2 Optimization of $NaBH_4$ concentration and sorbent amount

$NaBH_4$ is a reducing agent with wide application in chemistry. In this study, its multiple advantages were exploited: (a) reduction of non-fluorescent specie into fluorescent specie; (b) degradation of organic matrices and dyes [17] (c) better extraction recovery of KP by decreasing force of attraction between KP functional groups and serum contents (lipid, fats and hemoglobin) and by suppressing the ionization of readily ionisable functional groups [32]. The effect of $NaBH_4$ concentration on sorbent activity was observed by comparing KP fluorescent signals at respective different increasing $NaBH_4$ (0.02, 0.04, 0.06, 0.08, 0.1, 0.12, 0.14 and 0.16 M) concentrations. It was observed KP fluorescence gradually increases as the $NaBH_4$ concentration increased and it became maximum at its 0.1 M concentration. Probably, at this concentration the maximum KP reduced into fluorescent specie and RSDs values were also considerable at this $NaBH_4$ concentration as shown in Fig. S3 (a).

The sorbents (SnO_2 NPs) have special significance in this analytical method development as its dual functions were exploited; first for selective conversion of non-fluorescent KP into a fluorescent specie and second for selective adsorption of dyes, organic matter, metals and polar interferences as explained in introduction. Therefore, sorbent amount was gradually increased from 20, 40, 60, 80 and 100 µg in order to discover its optimum amount. It was observed the fluorescence intensity of KP dramatically increased when sorbent amount reached to 40 µg. Later on, there was no significance increase of KP fluorescent intensity was observed as shown in Fig. S3(c). In order to exploit it as sorbent along with as a reducing agent, a considerable amount of 60 µg was chosen and further used in the method validation.

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3.3.3 Optimization of agitation time

In order to have optimum fluorescent peak of KP, the selective reduction time of KP into fluorescent specie at 40 °C was monitored. It was observed the optimum peak area of KP was observed at 60 minute. Most probably this time reflects the maximum completion of NPs based catalyzed reaction of KP into fluorescent specie as shown in Fig. S4. In addition, this time is wide enough for selective degradation of dyes and organic matter in the presence of sun light [17].

3.4. Analyses of human serum, urine and canal water samples

The optimized method was applied for quantification of KP in three complex samples (human urine, serum and lake water). The clean IC separation and fluorescence detection of non-fluorescent KP in all three sample is portrayed in Fig. 7 (a-c).

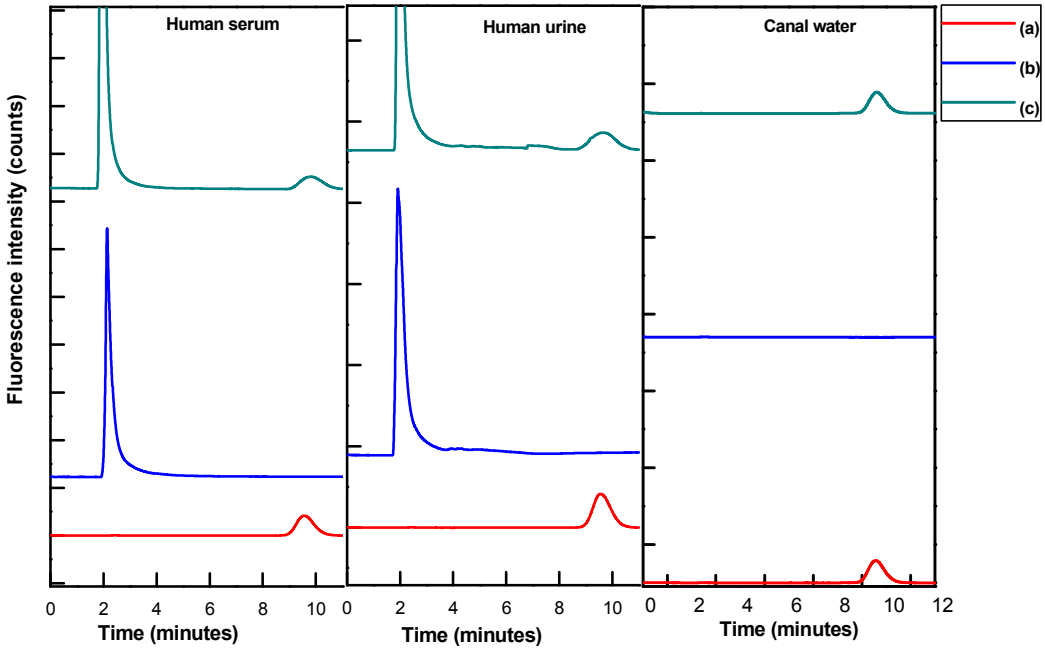

Fig. 7 Samples (human serum, urine and canal water) chromatograms spiked with KP (0.5 mg/kg) after extraction with new μ-sample extraction method. Column: IonPac® AG12A (50mm x 4 mm i.d; 13 mm particle size) and IonPac® AS12A (250 mm x 4 mm i.d; 13 mm particle size), eluent: 5 mM Na_2CO_3 with 10% (v/v) ACN at flow rate of 1.0 mL/min, fluorescence detection at $\lambda_{ex}/\lambda_{em}$ = 229 nm/292 nm. (a) Standard with sorbent treatment; (b) real sample without sorbent treatment and (c) spiked sample after sorbent treatment.

3.5. Method validation

The validation of the optimized method was conducted by studying important analytical parameters such as; linearity, limit of detection (LODs) and quantification (LOQs), intra-day and inter-day precision and accuracy.

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For calibration and linearity study, six levels of concentration (0.2, 0.33, 0.5, 1, 1.3 and 1.5 mg/kg) were prepared in 50% ultrapure water and acetonitrile mixture and injected in quintet. The method showed good linearity for KP in the concentration range from 0.2 to 1.5 mg/kg with coefficients of determination (r^2) greater than 0.990 as shown in (Fig. S4).

The proposed analytical method's limit of detection (LOD) and quantification (LOQ) of KP in three samples (human serum, urine and canal water) were calculated on the basis signal-to-noise ratio (S/N) value of 3 and 10, respectively. LOD and LOQ values in standard and three samples were found in the range of 0.1 to 0.3 µg/kg and LOQ values ranged from 0.5 to 1.17 µg/kg as given in Table 1 and 2.

Table 1. Calibration parameters for ketoprofen in standard solution (n = 5).

Analyte	Linear range (mg/kg)	Linearity	slope	LOD (µg/kg)	LOQ (µg/kg)	Retention time (min)
Ketoprofen	0.2-1.5	0.990	66977	0.31	1.05	8.6

The matrix effect (ME) was evaluated to observe the signal suppression/enhancement. The obtained results are shown in Table 2. In order to calculate the matrix effect, a set of blank human serum, urine and canal water samples were spiked before and after extraction at 0.50 and 1.3 µg/kg concentration level of KP and following equation was applied for their calculation.

ME (%) = (B-D/A -1)100%

A is the peak area of the KP obtained for the standard solution

B is the peak area obtained for the samples spiked with the KP (s) after extraction

C is the peak area obtained for the samples spiked with the KP (s) before extraction

D is the peak area obtained for the sample unspiked with the KP before extraction.

Recovery studies of optimized method were conducted in quintet, by fortifying blank human serum, urine and canal water samples with KP at two concentrations level 0.5 and 1.3 µg/ kg. The recovery results were obtained in the range of 85.1 to 101.1% as given in Table 2.

Table 2. Analytical parameters study for ketoprofen in human urine, serum and lake water samples (n = 5).

Sample	Add con. (µg/kg)	Precision		Recovery (%)	LOD (µg/kg)	LOQ (µg/kg)	Matrix effect
		Intra-day	Inter-day				
canal water	0.5	4.56	2.30	101.4	0.300	1.17	-0.190
canal water	1.3	10.50	16.30	91.6	0.310	1.40	1.000
urine	0.5	7.57	2.73	86.4	0.110	0.58	7.800
urine	1.3	6.87	7.60	85.1	0.100	0.49	-0.140
serum	0.5	11.30	2.30	101.4	0.390	1.30	-0.190
serum	0.5	8.71	16.30	91.6	0.300	1.10	1.000

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Both intra-day precision and recoveries were examined by analyzing spiked human serum, urine and canal water samples five times in one day at two spiked concentrations level of 0.5 and 1.3 mg/kg, while the inter-day precision was calculated by running the all three samples for two consecutive days at same two spiked concentrations level. Satisfactory results were obtained for the proposed method with RSDs values lower than 16.3%. Table 3 shows this novel analytical method has high sensitivity compare to previously used extraction and analytical techniques.

Table 3. Comparison of this method to other sample extraction method and analytical techniques for determination of KP.

No.	Samples	Extraction methods	Analytical techniques	LOD	References
1	Water samples	SPE	HPLC-UV	0.35 mg/L	33
2	Gel formulation	volumetric	HPLC-UV	0.20 mg/kg	2
3	Pharmaceutical dosage	direct injection	HPLC-UV	0.50 mg/kg	34
4	urine and plasma	SPE	HPLC-UV	0.29 – 0.39 mg/L	35
5	Plasma	SPE	RP-HPLC-UV	0.12 mg/L	36
6	Pharmaceutical formulation	direct injection	LC-APCI-MS	0.50 µg/L	12
7	Waste and river water	SPE	GC–MS	30-420 µg/L	9
8	Waste water	SPE	HPLC-*uv*-FLD	66 µg/L	14
9	Canal water, urine, serum	µ-extraction method	IC-FLD	0.1-0.3 µg/kg	This work

3.6. Significance of method

(a) Eliminates the need for pre- or post-column UV irradiation and electrochemical and other derivatization (no need for extra reagent, pumps, UV reactor etc.); (b) cost effective and green as minimum consumption of chemicals and reagents; (c) involved dual application of sorbent; (d) opening a new research gate for sensitive and selective determination of other non-fluorescent analytes; (d) good linearity, LOD and sensitivity.

4. Conclusions

This manuscript for the first time describes a novel dual application of SnO_2 nanoparticles in ion chromatography for selective conversion of non-fluorescent ketoprofen into highly fluorescent specie and as sorbent in a µ-sample preparation method for elimination and adsorption of matrix interferences in three complex samples (human serum, urine and canal water). This work also appeared as precise, sensitive, specific, accurate, cost effective and green (only 900 µl ACN in sample extraction) for the determination of KP in complex samples with no or minimum matrix effect.

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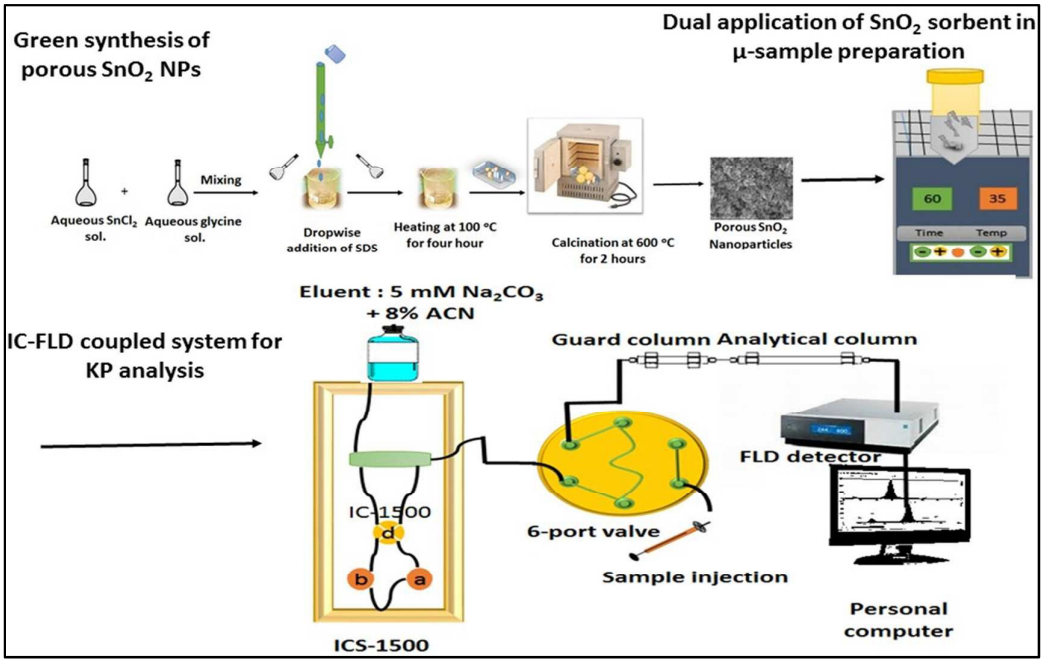

Systematic display of dual application of SnO_2 NPs in ion chromatography for selective determination of non-fluorescent KP in complex samples.