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Pharmacokinetics of ketorolac loaded to polyethylcyanoacrylate nanoparticles using UPLC MS/MS for its determination in rats

M.A. Radwan^{a,*}, B.T. AlQuadeib^b, N.M. Aloudah^a, H.Y. Aboul Enein^c^a Department of Clinical Pharmacy, College of Pharmacy, King Saud University, PO Box 22452, Riyadh 11495, Saudi Arabia^b Department of Pharmaceutics, College of Pharmacy, King Saud University, PO Box 22452, Riyadh 11495, Saudi Arabia^c Pharmaceutical and Medicinal Chemistry Department Pharmaceutical and Drug Industries Research Division National Research Centre, Dokki, Cairo 12311, Egypt

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ABSTRACT

Polyethylcyanoacrylate (PECA) nanoparticles (NPs) have been employed as biodegradable polymeric carriers for oral (PO) delivery of ketorolac. The nanoparticles were prepared by polymerization technique at room temperature in a continuous aqueous phase at pH 2.5. This polymerization technique was able to hold 76–96% of ketorolac and the drug loading was a monomer concentration dependent. The feasibility of PECA NPs as PO controlled drug delivery systems of ketorolac was investigated in two groups of rats which were given orally either ketorolac tromethamine solution (1.5 mg/kg) or the selected ketorolac NPs aqueous dispersion (1.6 mg/kg). Ketorolac plasma concentrations were measured by a new fully validated specific, precise and accurate ultra-performance liquid chromatography tandem mass spectrometry (UPLC MS/MS) assay. The detection was performed on Waters TQ detector via negative electrospray ionization in a multiple reaction monitoring mode. Linear response ($r^2 \geq 0.995$) was observed over the range of 10–10,000 ng/ml of ketorolac, with the lower limit of quantification of 5 ng/ml with 1 μ l injection volume. The intra- and inter-day precision (relative standard deviation, R.S.D.) values were <10% and the accuracy (relative error) was ≤ 8 for ketorolac concentrations. The drug solution is rapidly absorbed, distributed, and eliminated and shows a monophasic elimination phase. The assay was sensitive to follow pharmacokinetics of ketorolac in rats up to 24 h after a PO dose of its aqueous solution or NPs suspension. After NPs administration the mean C_{max} , 5.0 ± 1.3 mg/l, was attained at 1 h. The drug was successfully maintained around this elevated plasma drug concentration up to 6 h ($>2t_{1/2}$), in rats. The AUC was significantly higher after the NPs suspension than the solution of ketorolac. This present study provides evidence that the sorption of ketorolac to PECA NPs could control the drug release/elimination in rats.

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1. Introduction

Biodegradable polymeric nanoparticles (NPs) have been used frequently as drug delivery systems (Letchford and Burt, 2007) due to its grand bioavailability, better encapsulation, control release and less toxic properties. Poly (alkyl cyanoacrylate) (PACA) have been studied extensively as the carrier of different drugs and have succeeded in development of the nanoparticulate formulations for intravenous administrations (Barratt, 2000; Radwan, 2001a; Radwan and Aboul-Enein, 2002). Different research teams have published reviews about the NP formation mechanisms (Soppimath et al., 2001; Vauthier et al., 2003; Krauel et al., 2004), the classification of nanoparticulated systems (Vauthier et al., 2003; Letchford and Burt, 2007) and the techniques for preparation of NPs (Stella et al., 2007; Vauthier and Bouchemal, 2008; Vauthier et al.,

2008) which proves that PACA NPs are quite satisfied to be one of the drug delivery carriers to be investigated for more drugs.

Ketorolac is a non-steroidal anti-inflammatory drug (NSAID), with a potent analgesic and anti-inflammatory activity due to prostaglandin related inhibitory effect of drug. It is a non-selective cyclooxygenase (COX) inhibitor; it is used clinically for the management of postoperative and cancer pain. Ketorolac (free acid) is sparingly soluble in water and, therefore, it is marketed in the form of tromethamine salt (KT), which increases its solubility in water, for oral, intramuscular, intravenous administrations and as a topical ophthalmic solution. Previous studies have suggested that ketorolac's analgesic efficacy may be greater than that of other NSAIDs and comparable with that of morphine in models of acute pain (Yee et al., 1986; Brown et al., 1990; Goodman, 1991). Its therapeutic advantages over opioid analgesic drugs are that it is non-sedating, it is not associated with pruritis, nausea, respiratory depression or alteration of the carbon dioxide (CO₂)-minute ventilation response curve, urinary retention or ileus, and it has opioid-sparing qualities. Like other NSAIDs, however, ketorolac has been implicated as

* Corresponding author.

E-mail address: mradwan@ksu.edu.sa (M.A. Radwan).

a contributing cause of increased postoperative bleeding (Rusy et al., 1995), renal failure, and gastritis (Corelli and Gericke, 1993). The severity of these side effects is probably dose-related (Hyers et al., 1992; Green et al., 1996). Regardless of the route of administration, the biological half-life of the ketorolac ranges from 4 to 6 h (Bhaskaran and Suresh, 2004) in human.

The frequent occurrence of gastrointestinal disturbances including gastrointestinal bleeding, perforation and peptic ulceration along with the short mean plasma half-life has prompted for the development of various formulation strategies for the appropriate delivery of KT (Sinha et al., 2009). Gupta et al. (2000) were interested in polymeric micelles as ocular preparation, Sinha and Trehan (2005) prepared drug-loaded polycaprolactone and poly lactic-co-glycolic acid microspheres, Alsarra et al. (2005) investigated proniosomes as a potential drug carrier for transdermal delivery, Rokhade et al. (2006) developed semi-interpenetrating polymer network microspheres of gelatin and sodium carboxymethyl cellulose and recently, Genç and Jalvand (2008) produced controlled release hydrophilic matrix tablets.

At present, a great deal of emphasis is being placed on the development of controlled or sustained release forms for the drug as this would aid in achieving the required therapeutic efficacy and better tolerance with fewer gastrointestinal side effects. However, to our knowledge no team has investigated cyanoacrylate nanoparticles as a drug carrier for KT or utilized UPLC MS/MS for ketorolac determinations in plasma. Therefore, the main objective of this study was to develop ketorolac submicroscopic polymeric delivery system using polyethylcyanoacrylate (PECA). Furthermore, the capability of the developed oral PECA delivery system to release ketorolac in a controlled manner in rats was also studied using a new developed UPLC MS/MS assay. Both the oral solution and NPs dispersion of ketorolac PECA were studied in rats.

2. Materials and methods

2.1. Materials

Ketorolac tromethamine, flurbiprofen, HPLC grade formic acid and ammonium formate were purchased from Sigma–Aldrich (St. Louis, MO, USA). Pluronic F68 Prilled was obtained from Ruger Chemical Co. Inc., Irvington, NJ. All other reagents and chemicals were analytical grade, and were used as received.

2.2. Nanoparticles preparation

NPs were prepared by polymerization technique in continuous aqueous phase according to a previously described method (Radwan, 2001b) with some modification preparation optimization. Briefly, the ethylcyanoacrylate, monomer, solution (200 μ l) was slowly added, using mechanical stirring, to a 4 ml of 2×10^{-3} M HCl (pH 2.5), 0.5% (w/v) dextran 70 and 0.5% (w/v) pluronic F68. Polymerization was carried out at room temperature without any addition of a chemical initiator. After 15 min of the addition of the monomer, KT solution (1 ml) was added drop wise. After 1.5 h, the resulting milky suspension was neutralized with 0.01 M NaOH and glucose (5%, w/v) solutions. Each batch was prepared in triplicate.

2.3. Determination of ketorolac loading

The sorption of ketorolac on PECA NPs was measured immediately after preparation of the spheres. The suspension was centrifuged at 20,000 rpm for 30 min, and filtered through 0.22 μ m filter. The sorption of ketorolac on PECA NPs was assessed in the filtrate using a UPLC MS/MS. NPs in the sediment were freeze-dried at -70°C . The lyophilized NPs were weighed before storage at 5°C .

Ketorolac content in the NPs was determined by UPLC MS/MS after dissolving 5 mg of the NPs in 0.5 M NaOH solution.

2.4. Nanoparticles characterization

Scanning electron microscopy (Joel Scanning Microscopy, JSM6060 LV, Tokyo, Japan) was used to study the size and morphology characteristics of NPs.

2.5. In vivo study: animal dosing and sampling scheme

Animals handling was fully complied with our institutional policies. Twenty four male Wistar rats (175–230 g) were used in this study. Rats were randomly divided into 2 groups ($n=12$) for KT solution or NPs suspension and each group is divided into two subgroups ($n=6$) for different sampling time. Each subgroup was marked and housed in one cage. Animals were fasted for about 12 h prior to experiments but water was available *ad libitum* at all times. For the first group, KT powder (14.7 mg) was dissolved in 2 ml sterile water for injection (SWFI) and each rat received an oral dose of 1.5 mg/kg of ketorolac using rats oral tubing connected to insulin syringe (1 ml). While for the second group an aliquot of ketorolac NPs containing 10 mg of ketorolac was suspended in SWFI and each rat was given equivalent to 1.6 mg/kg of ketorolac as described above but the NPs suspension was vortexed for few seconds before each administration. Blood samples (0.5 ml) were collected in 0.6 ml graduated microtainer, containing lithium heparin, at 0, 0.5, 2, and 4 h and at 1, 6, 8, 12 and 24 h from the first and the second subgroups, respectively, after drug administrations. Blood samples were collected from the orbital venous plexus, under light halothane anesthesia. Four to five blood samples were collected from each rat per day to avoid any damage to the eye. Therefore, each data point is the mean of 6 replicates. Plasma samples were separated by centrifugation at 3000 rpm for 15 min and stored at -20°C till assayed.

2.6. Chromatographic system and conditions

The analysis was carried out on a Waters ACQUITY UPLC™ system with cooling autosampler and column oven. An ACQUITY UPLC™ BEH C8 column (50 mm \times 2.1 mm, 1.7 μ m (Waters Corp, Milford, MA, USA) was employed for separation with the column temperature maintained at 40°C . The gradient elution for UPLC analysis consisted of two solvent compositions: Solvent A: 5 mM ammonium formate and 1% acetonitrile in water (pH 3) and solvent B: 0.2% formic acid in acetonitrile. The gradient began with 80.0% eluent A and changed linearly to 20% A within 3.5 min, and changed back to 80% A within 0.1 min. Throughout the UPLC process the flow rate was set at 0.4 ml/min and the run time was 4 min.

A Waters TQD™ tandem quadrupole mass spectrometer (Waters Corp., Milford, MA, USA) equipped with an electrospray ionization (ESI) interface was used for analytical detection. The ESI source was set in negative ionization mode. Quantification was performed using MRM of the transitions of m/z 254.10 \rightarrow 209.9 for ketorolac and m/z 243.10 \rightarrow 199 for flurobiprofen (IS), with scan time of 0.10 s per transition. The optimal MS parameters were as follows: capillary voltage 3.0 kV, cone voltage 20 V, source temperature 120°C and desolvation temperature 400°C . Nitrogen was used as the desolvation and cone gas with a flow rate of 500 and 4 L/h, respectively. Argon was used as the collision gas at a pressure of approximately 0.3 Pa. The optimized collision energy for ketorolac and IS was 15 eV. All data collected in multi-channel analysis (MCA) mode were acquired and processed using MassLynx™ V 4.1 software with QuanLynx™ V 4.1 program (Waters Corp., Milford, MA, USA).

2.7. Preparation of ketorolac standards and quality control samples

Stock standard solutions of KT and IS were prepared in methanol at a concentration of 0.5 mg/ml and stored in 4 ml amber glass vials at -20°C . Different working standard solutions of ketorolac (0.01–25 $\mu\text{g/ml}$) and the IS (1 $\mu\text{g/ml}$) were prepared by dilution of the abovementioned stock solutions in water:acetonitrile 50:50 (v/v) and were kept refrigerated in amber vials at -20°C . The solutions of KT and the IS were stable for at least 1 month, under the described conditions. The plasma calibrations standards were prepared in replicate ($n=6$) at concentrations of 10, 20, 50, 100, 500, 1000, and 5000 ng/ml by spiking appropriate aliquots of KT working solutions and 20 μl of the IS to 200 μl of blank rat plasma. Low, medium and high concentration quality control (QC) samples at concentrations of 10, 100 and 5000 ng/ml of KT with 100 ng/ml of IS were prepared. The spiked samples were then treated as described in the plasma sample preparation section below.

2.8. Plasma sample preparation

Aliquot of plasma samples collected from different rats were subjected to protein precipitation as follows. Samples aliquot of 200 μl (or a calibration standard or a QC sample) and 20 μl of IS working solution were added to a 1.8 ml Eppendorf tube and the mixture was vortexed for 10 s. Then, 800 μl of acetonitrile were added and the mixture was vortexed for 1 min followed by centrifugation at 20,000 rpm for 15 min at 10°C . The supernatant was transferred into a clean glass tube and evaporated to dryness under a gentle stream of nitrogen. The residue was reconstituted in 100 μl of water:acetonitrile (50:50, v/v), vortexed for 1 min, centrifuged at 3000 rpm for 5 min, transferred into a plastic autosampler vial with pre-slit septum (Waters, USA) where 1 μl was injected into the UPLC MS/MS system.

2.9. Ion suppression study

The absence of ion suppression was demonstrated by the method of Matuszewski et al. (1998). Six different batches of drug-free rat plasma were extracted without any drug or IS added as abovementioned. The extracts were reconstituted with ketorolac at three nominal concentrations 10, 100, and 5000 ng/ml (low, medium, high). The peak areas of the samples were compared to that of the unextracted reference standard solutions containing the equivalent nominal amount of ketorolac in the mobile phase ($n=6$). The mean area ratio (reconstituted extracts/reference solutions) were 0.95 for ketorolac with R.S.D. of <3.6%. Thus, no ion-suppression was observed.

2.10. Method validation

The method was validated for selectivity, linearity, precision, accuracy, carry over, extraction recovery and stability according to the accepted guidelines of FDA (2001) and USP XXXIII (2003) of bioanalytical methods.

The selectivity of an analytical method is its ability to measure accurately an analyte in the presence of endogenous compounds. Therefore, six blank, drug-free, plasma samples obtained from six different rats were analyzed according to the procedure described above. The corresponding chromatograms were tested for possible interferences at the retention times (RT) of ketorolac and the IS.

The specificity of the method was investigated by comparing the chromatogram of blank plasma spiked with standard solutions to the samples collected from rats after KT administration.

The intra-day precision and accuracy was determined within 1 day by analyzing six replicates of the QC samples at concen-

trations of 10, 100, and 5000 ng/ml of ketorolac. The inter-day precision and accuracy was determined on 3 separate days. The intra- and inter-day precision was defined as the relative standard deviation (R.S.D.). The accuracy was presented as percent relative error, R.E. [(measured concentration – nominal spiked concentration)/nominal spiked concentration] \times 100.

Extraction efficiency was determined for the QC samples at the three concentrations levels (low, medium, high) with those of post-extraction spiked blank plasma samples. The absolute extraction recoveries were calculated by comparing the peak areas of the samples to that of the unextracted standard solutions containing the equivalent amount of ketorolac ($n=6$).

The nominal value of ketorolac concentration (ng/ml) in plasma was plotted as a function of the peak area ratio obtained of ketorolac and the IS. The day curve was accepted, if the relative standard deviation (R.S.D.) was $\leq 10\%$ for all the tested concentrations (low, medium and high). The limit of detection (LOD) was defined as the lowest concentration of the analyte resulting in a signal-to-noise ratio of 3:1. The lower limit of quantitation (LLOQ) was defined as the lowest drug concentration which can be determined with a R.S.D. $\leq 20\%$ and an accuracy of $100 \pm 20\%$ on a day-to-day basis. Accuracy and precision at the LLOQ were estimated.

The robustness of the method was determined, by using two different ACQUITY UPLCTM BEH C8 columns (50 mm \times 2.1 mm, 1.7 μm).

Freeze–thaw stability of the plasma samples was evaluated by exposing quality control samples to three freeze (-20°C)–thaw (room temperature) cycles before sample preparation. The stability of the samples in autosampler was evaluated by analyzing extracted quality control samples after being placed in the autosampler at 20°C for 24 and 72 h.

3. Data and statistical analysis

All *in vitro* results were expressed as the mean \pm SD of six replicates. The results were calculated by linear regression without weighing, using the equation: $Y = a + bX$, where Y is the area under the peak (AUP) ratio of the drug to the internal standard, a is the intercept, b is the slope, and X is the concentration of ketorolac. The R.S.D.% was calculated for all values. The *in vivo* results were presented as the mean \pm S.E. of six replicates. Pharmacokinetic parameters were estimated using model-independent methods (Gibaldi and Perrier, 1982). The terminal elimination rate constant (λ_n) was estimated by linear regression analysis of the terminal portion of the log-linear blood concentration–time profile of a drug. The terminal elimination half-life ($t_{1/2}$) was calculated from the terminal elimination rate constant using the formula $t_{1/2} = 0.693/\lambda_n$. The mean peak drug concentration (C_{max}) and the time to reach C_{max} (T_{max}) were derived directly from the individual blood levels. The area under each drug concentration time curve (AUC, $\mu\text{g ml}^{-1} \text{h}$) to the last data point were calculated by the linear trapezoidal rule and extrapolated to time infinity by the addition of $C_{\text{Last}}/\lambda_n$ where, C_{Last} is concentration of the last measured plasma sample. The apparent body clearance (Cl/F) was calculated using the equation $\text{Cl/F} = \text{Dose/AUC}$.

The student t -test was used to examine the concentration difference at each day, and one-way analysis of variance (ANOVA) was employed to evaluate the reproducibility of the assay using SPSS Statistics 17 (2008). The level of confidence was 95%.

4. Results and discussion

4.1. PECA nanoparticles

Scanning electron microscopy of ketorolac prepared NPs (Fig. 1) showed uniform smooth spherical shape with narrow distribu-

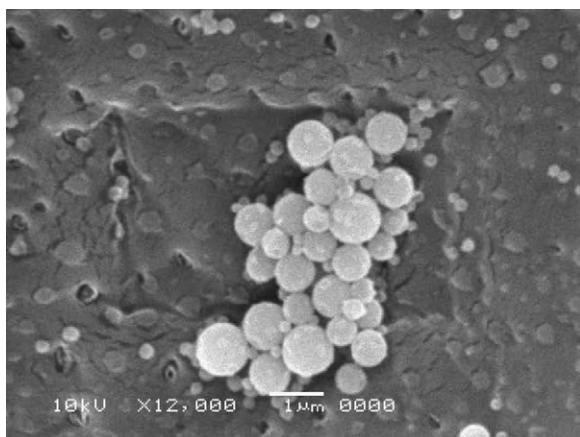


Fig. 1. Electron scanning micrograph of ketorolac-loaded PECA NPs showing the spheres mean diameter of <1000 nm.

tion (a mean diameter < 1000 nm) which was in agreement with literature (Couvreur et al., 1979). It was found that the addition of ketorolac 15 min after initiation of polymerization resulted in a substantial increase (from 15 to 93%) in drug loading. This polymerization technique was able to hold 76–96% of ketorolac added 15 min after initiation of polymerization. Therefore, this time (15 min) was selected for the addition of ketorolac throughout this study after initiation of polymerization. The percentage drug loading was a monomer concentration dependent which is in agreement with our previous finding (Radwan, 2001b). The pluronic F68 existence in the polymerization medium of PECA NPs improved the drug adsorption at all tested times. The drug loading percentage in the selected PECA NPs formulations was 8.4 ± 1.2

which is comparable to our previous finding of 9.2 ± 0.5 for metoclopramide loaded to PECA NPs (Radwan, 2001b).

4.2. Chromatography

UPLC MS/MS extracted chromatograms are shown in Fig. 2 of ketorolac after 24 h (A) of NPs suspension oral administration to a rat and MRM transitions of IS and ketorolac (B and C, respectively). The retention times were approximately 1.6 min and 2.4 min for ketorolac and IS, respectively. The peaks of ketorolac and flurbiprofen were well separated without any significant interference from any endogenous substance at the retention time of ketorolac or the IS within the 4 min run time. During the 3 months of validation, there was no significance change in the observed retention times of ketorolac or the IS (R.S.D. < 0.8%).

4.3. UPLC MS/MS validation

No endogenous plasma components eluted at the retention times of ketorolac or IS, of the six different rat plasma, which proves the assay specificity.

Excellent linear relationships ($r^2 > 0.995$) were demonstrated between AUP ratio of ketorolac to the IS in rat plasma over the studied concentration ranges. The LOQ of this assay was 5 ng/ml in rat plasma with the corresponding R.S.D. of 12.6%, and the LLOD was 1 ng/ml at a signal-to-noise ratio of >3, with injection volume of 1 μ l.

The mean correlation coefficient was > 0.994 and the R.S.D. of the slopes of the three standard plots was 10.2%. Analysis of variance of the data indicated no significant difference ($p > 0.05$) in the slopes, intra- and inter-day, of the calibration curves. The results confirmed the reproducibility of the assay method.

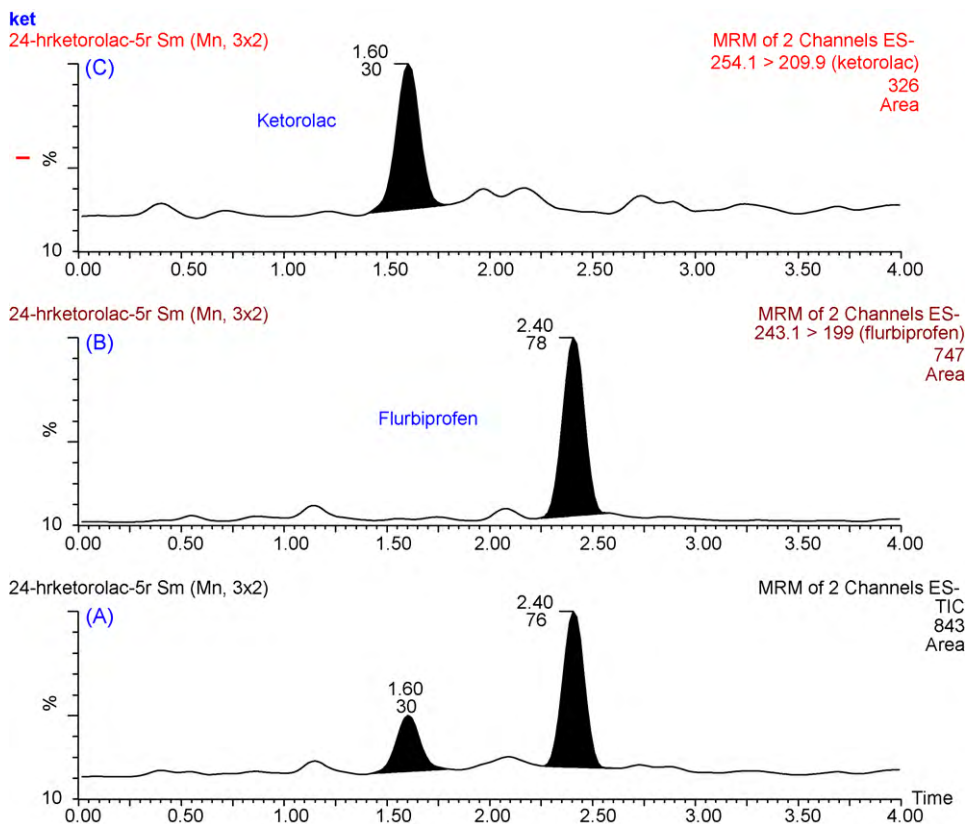


Fig. 2. UPLC MS/MS extracted chromatograms of ketorolac after 24 h of NPs suspension oral administration of 1.6 mg/kg to a rat (A) and MRM transitions of flurbiprofen (IS) and ketorolac ((B) and (C), respectively).

Table 1Precision (R.S.D.), accuracy (relative error) and recovery for determination of ketorolac in spiked rat plasma samples ($n = 6$).

Nominal concentration (ng/ml)	Recovery (%)	Intra-day ^a precision		Inter-day ^b precision	
		Accuracy (%)	R.S.D. (%)	Accuracy (%)	R.S.D. (%)
10	106.4	-5.3	9.5	6.6	8.7
100	101.7	4.4	7.5	7.9	9.6
5000	91.2	6.3	8.7	-7.5	6.7

^a The analyses were performed in the same day; R.S.D.: relative standard deviation.^b The analyses were performed in 3 different days within 3 months.

The mean percentage recovery of ketorolac was 93.5 ± 8.0 with R.S.D. of ≤ 10 . There was no significant difference in the extraction efficacy of the present assay over the range of concentrations studied. The accuracy and precision results are shown in Table 1. Precision is represented as R.S.D. and accuracy was calculated as relative error (R.E.). The intra-run and inter-run precision (R.S.D.) was $\leq 10\%$ and accuracy as R.E. was $< 8\%$.

Ketorolac and IS were both stable in processed samples held in the autosampler at 20°C for at least 24 h with mean calculated values within 6.4% of the nominal concentration. There was no evidence of sample carry-over from run to run. In addition, the matrix effect assessed by spiking samples post-processing showed $< 8\%$ difference from spiked injection solvent.

4.4. In vivo performance of ketorolac NPs

Ketorolac plasma concentration–time profiles after PO administrations of both KT solutions and NPs suspension are depicted in Fig. 3. Ketorolac plasma concentration (mean + S.E.) time profiles in rats after PO administrations of 1.5 mg/kg drug solution (keto-solution) and 1.6 mg/kg of drug-loaded NPs suspension of PECA (keto-nano). The pharmacokinetic parameters for the solution and NPs dosage form are summarized in Table 2. After PO administration of KT solution, the drug was rapidly absorbed, distributed, and eliminated. The C_{max} (4417.5 ± 2919.8 ng/ml) was achieved after 1 h of the administration. The assay was able to detect the drug concentrations up to 24 h after oral dosing. The MRT was 4.4 h, and the apparent CI was 0.072 (L/h)/kg.

After drug-loaded NPs administration (1.6 mg/kg), there was a rapid release followed by a slower one. Ketorolac concentration

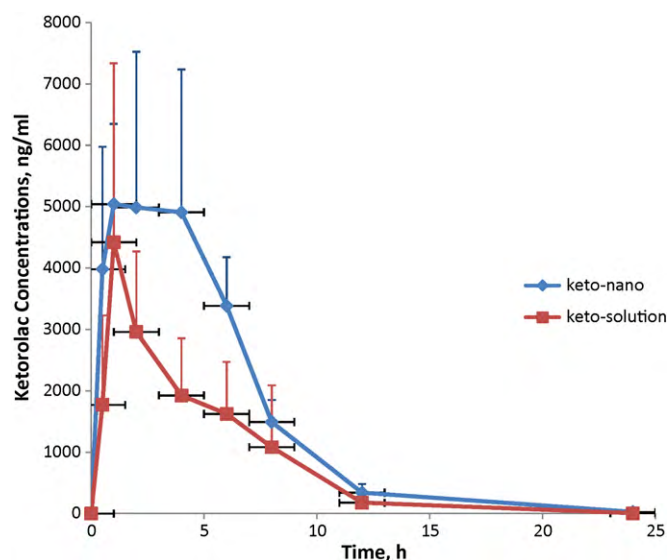


Fig. 3. Ketorolac plasma concentration (mean + S.E.) time profiles in rats after PO administrations of 1.5 mg/kg drug solution (keto-solution) and 1.6 mg/kg of drug-loaded NPs suspension of PECA (keto-nano) ($n = 6$).

Table 2Pharmacokinetic parameters of ketorolac following an oral administrations of ketorolac solutions and ketorolac loaded to polyethylcyanoacrylate nanoparticles in rats ($n = 6$).

Parameters	Ketorolac solution	Ketorolac nanoparticles
Dose, mg/kg	1.5	1.6
$AUC_{(0-t)}$, (mg/l)h	20.40	37.2
AUC, (mg/l)h	20.99	38.6
C_{max} , ng/ml (\pm S.E.)	4417.5 (2919.8)	5040 (1306)
T_{max} , h	1	1
$AUMC_{(0-t)}$, (mg/l)h ²	91.26	171.92
AUMC, (mg/l)h ²	91.68	174.93
Cl/F, (L/h)/kg	0.072	0.042
$t_{1/2}$, h	2.12	2.84
MRT, h	4.37	4.53

of 3976 ± 1999.7 ng/ml was achieved at 0.5 h. This rapid release of ketorolac could be due to the initial surge of the drug (burst release) from the surface of the NPs in plasma. The drug concentration was increased to 5039.6 ± 1306.4 ng/ml, C_{max} , after 1 h (T_{max}). The drug was successfully maintained around this elevated plasma drug concentration up to 6 h in rats. After 8 h, ketorolac loaded to NPs concentration was greater by 38% than that after the solution with dose normalization. The mean AUC after NPs was 72% higher than that after the solution which indicates that NPs decreased the apparent CI of ketorolac since the drug absorption was not affected by NPs formulations as shown earlier. NPs administration was not significantly change the MRT than that of the KT solution which was not in agreement was our previous finding with metoclopramide (Radwan, 2001b) which could be due to the difference in solubility between the two drugs. Previous reports have shown that nanoparticles as colloidal carrier are mainly captured by reticuloendothelial tissues (Couvreux et al., 1980; Kreuter and Hartmann, 1983; Iium et al., 1984). Ketorolac were captured by the reticuloendothelial tissues where it slowly released and cleared within the first few hours before the complete degradation of the PECA NPs. Therefore, there was no significant change in the elimination $t_{1/2}$ or the MRT of ketorolac after NPs oral administration. Even though the prescribed formulation showed a significant increase in the AUC and more extended release than the solution, more work is needed for optimization of ketorolac formulation for better pain management in patients.

5. Conclusions

The present study provides evidence that the sorption of ketorolac to PECA NPs is a successful attempt to maintain, up to 6 h ($> 2t_{1/2}$), elevated plasma drug concentrations of ketorolac in rats. Also in this report a specific and sensitive UPLC MS/MS method for the determination of ketorolac concentrations in rat plasma was described. Chromatographic conditions have been optimized to be simple and rapid for sample preparation and chromatography. In fact, the sample pretreatment procedure to quantify the drug in plasma required a simple precipitation of proteins with acetonitrile. This described UPLC MS/MS method was found suitable for the analysis of plasma samples of oral solution of KT or the developed PECA NPs in rats.

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