BioNanoScience (2020) 10:745–757
https://doi.org/10.1007/s12668-020-00754-w

Comparison Between the Pharmacokinetics Data of Ketorolac Tromethamine Wafer a Novel Drug Delivery System and Conventional Ketorolac Tromethamine Tablets to Enhance Patient Compliance Using a New LC-MS/MS Method

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Published online: 26 May 2020

Abstract
Ketorolac tromethamine (KTM) is a potent and widely used non-steroidal anti-inflammatory drug. Despite its efficacy, it causes gastric irritation and increases the risk of gastrointestinal injuries. This study aimed to formulate KTM wafer to overcome its harmful gastrointestinal side effects. By solvent evaporation method six formulae prepared with different concentrations of polymers of sodium carboxymethyl cellulose, sodium alginate, and hydroxypropyl methylcellulose (HPMC E15). The formula F2 with high concentration of sodium alginate wafer, shows disintegration time in 85 s, with pH 6.6,% drug loaded with 102% and high dissolution release rate in 20 min. Drug release pattern appears to be second order. The mean C_{pmax} values of F2 wafer and the marketed product were 2135.47 ± 13.83 ng/mL and 1073 ± 23.5, respectively. The median values of T_{max} were 1 and 3 h, respectively. The calculated AUC0 − ∞values were 2087 ± 71.58 and 3981 ± 62.34 ng h/mL for F2 and marketed product, correspondingly. The relative bioavailability was found to be 0.52. A new rapid, sensitive, and specific LC-MS/MS fully validated method was developed for the determination and quantification of KTM, using torsemide as internal standard, in biological sample. It was successfully applied to perform the pharmacokinetic and the bioavailability study.

Keywords Lyophilized wafer · Ketorolac tromethamine · Pharmacokinetics · LC-MS/MS · Bioanalytical validation

1 Introduction

Buccal delivery of drugs offers rapid absorption and high bioavailability. It is a widely acceptable drug delivery system to patients. Lyophilized wafers placed on the patient's tongue absorb saliva quickly and disintegrate to release the drug within seconds [1]. At all the times, there are rising hassle for developing a dosage form which improve patient convince and compliance particularly for oral/buccal drug delivery system. Due to small size, little dose, thickness of buccal wafer over other dosage form is most acceptable and pleasant. Lyophilized oral wafer drug delivery system is an alternative approach for the tablets, capsules, and liquid oral dosage forms for pediatric and geriatric patients. Lyophilized wafer possesses relatively larger surface area compared with the other dosage forms which ensures greater patient compliance, especially in geriatrics and pediatrics An ideal buccal wafer should be flexible, elastic, and soft; moreover, it must possess good bioadhesive properties so that it can remain in the oral cavity for the pre-determined period [2, 3].

The advantages of using this technology include rapid drug release, direct systemic effects, and avoiding first-pass metabolism [4, 5].

Noteworthy, a buccal dosage form can release drug to the oral cavity and promote absorption throughout the gastrointestinal tract; due to highly porous solid matrixes, these systems disintegrate rapidly in the mouth and increase therapy efficacy for disorders that require fast intervention [6]. Buccal

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delivery provides easy access to highly vascularized tissue, avoiding first-pass metabolism and concomitant liquid intake. Furthermore, the neutral environment of the mouth allows for administration of acid-sensitive active pharmaceutical ingredients (APIs) [7]. For instance, acute attacks of migraine often come with nausea, which implicate in parenteral medication to avoid vomiting. With the advent of Rizatriptan wafers, pain decreases after around 20–30 min of drug administration, like standard subcutaneous sumatriptan [8]. To inhibit nausea and vomiting of migraine attacks and other medical conditions, fast-disintegrating antiemetic versions gained wide acceptance, including ondansetron and domperidone. Oral ondansetron was as efficacious as its intravenous administration in prevent emesis after laparoscopic cholecystectomy [9]. Ketorolac/lidocaine polymeric wafers reduced pain and enhanced tissue healing in dental patients previously subjected to gingivectomy [10]. Wafer formulations are appropriate in allergenic conditions, cough and cold remedies, sore throat, nausea, pain, and CNS disorders [11].

Sodium alginate (SA) and carboxymethylcellulose (CMC) are major commercial polysaccharide polymers and easily available. This is due to their advantages including low cost, biocompatibility, and biodegradability [12]. They are physically stable under normal conditions. They are chemically inert to the active ingredients, compatible with packing components and easily available [13]. Hydrophilic sodium carboxymethyl cellulose delivers drugs effectively through gastrointestinal mucosal tissue absorption, does not require organic solvents, and is usually combined with other matrix formers.

However, it has limited application because it is insoluble in water as well as in common organic and inorganic solvents. Sodium carboxymethyl cellulose (CMC) is a modified cellulose obtained through its carboxymethylation. CMC has an excellent film-forming ability, is biodegradable, and has low toxicity. Moreover, it has strong hydrophilicity and a stable internal network structure. These properties can be used to improve the performance of composite films. Sodium alginate (SA) is a type of biopolymer that is extracted from brown seaweed. Because of its wound-healing properties, good moisture absorption and permeability, high viscosity in aqueous solutions, and other characteristics, SA has been widely used in biomedical applications and for fabricating new coatings [14, 15]. SA films have high mechanical strength; however, its poor moisture resistance hinders their wide application [16].

Hydroxypropyl methyl cellulose (HPMC) is non-ionic cellulose ether made from natural cotton fiber under series of chemical processing. It is odorless, tasteless, and non-toxic white powder and can be dissolved in cold water to form a transparent viscous solution with the properties of thickening, binding, dispersing, emulsifying, film coating, suspending, absorbing, gelling, water retention and colloid protection. Hydroxypropyl methylcellulose has better enzymatic resistance than methylcellulose, and its solution is less likely to be enzymatically degraded than methylcellulose.

Lyophilization technique produces wafer with greater porosity, allowing for shorter disintegration times than compressed oral fast-dissolving film [17]. Freeze-dried formulations have low water content and do not support microbial growth, precluding the need for inclusion of these additives.

Ketorolac tromethamine (KTM) is a non-steroidal anti-inflammatory drug (NSAID) commonly used for relief of acute pain before or after medical procedures. It works by blocking the synthesis of prostaglandins thereby reducing inflammation, swelling, pain, and fever. The analgesic potency of KTM is comparable with morphine, but without the opiate receptor-associated side effects. The beneficial effects of ketorolac are probably due to its ability to block prostaglandin synthesis by preventing the conversion of arachidonic acid to the endoperoxides. Animal studies demonstrated that the analgesic activity of ketorolac results principally from a peripheral action [18].

So, the aim of the present work is to develop a lyophilized wafer of KTM, comparing the pharmacokinetic parameters with the conventional tablet present in the market. An advanced LC-MS/MS method created and validated for the identification and quantification purpose of KTM in plasma to perform pharmacokinetic and bioavailability studies.

2 Experimental

2.1 Materials

Ketorolac tromethamine (KTM) (Fig. 1a) was obtained as a gift sample from Epico Pharma Company, Cairo (Egypt), while torsemide the internal standard (IS) (Fig. 1b) was provided from Sigma-Aldrich, Germany. Sodium alginate, hydroxypropyl methylcellulose (HPMC E15), and carboxymethyl cellulose (CMC) were from Dow Wolff Cellulosics GmbH, Bomlitz, Germany. Acetonitrile (HPLC grade) and ammonium formate (for Mass Spectrometry), ammonium acetate, disodium hydrogen phosphate, and potassium dihydrogen phosphate of analytical grade were purchased from Sigma-Aldrich, USA. Propylene glycol was from Parchem Fine & Specialty Chemicals, USA, and mannitol from Roquette, France. All other chemicals or solvents were of analytical grade.

2.2 Instrumentation

For centrifugation, centrifuge (Eppendorf 5804 R, Hamburg, Germany) was used. As for mixing, vision scientific vortex mixer (KMC-1300V, China) was employed. The analysis was carried out on high-performance liquid chromatography

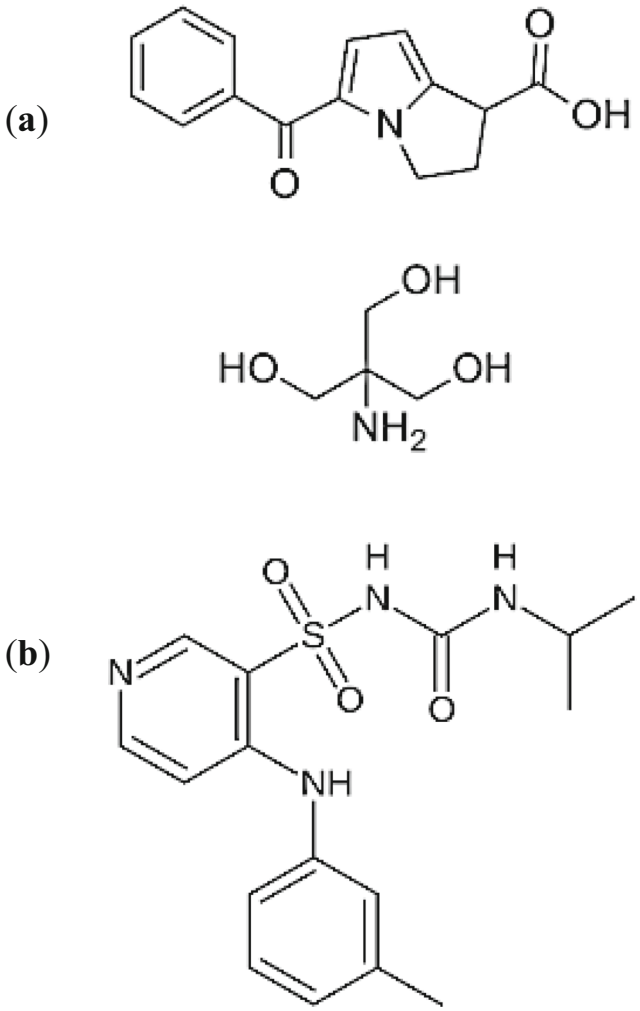

Fig. 1 Chemical structures of ketorolac tromethamine (KT) **a** and torsemide, the internal standard **b**

(HPLC) Shimadzu system (Shimadzu, Japan) equipped with solvent delivery unit (LC-20AT), an auto-sampler (SIL-20A), and coupled with mass spectrometer detector (API 3200 LC/MS/MS system, AB Sciex, Toronto, Canada) operating with nitrogen generator (N300DR peak scientific, Scotland). The separation was performed on Eclipse Plus, Agilent C18 column (3.5 μm, 50 × 4.6 mm dimensions). The software employed for data collection and handling was Shimadzu Chromatography Data System. While the data acquisition and data integration were performed using Analyst 1.4.3 software version (Applied Biosystems, MDS, SCIEX, Canada).

2.3 KTM Drug-Sodium Alginate Polymer Interaction

The incompatibility between the drug, hydroxyl methyl cellulose, and sodium alginate was characterized by Fourier transform infrared spectroscopy (FT-IR). The IR spectrum of pure drug, pure sodium alginate, and mixture containing both is compared with each other.

2.4 Designing of KTM Lyophilized Wafer

Six formulae of KTM were prepared from two different concentrations of each polymer 2% and 6%. The polymer was dissolved in quantity sufficient of distilled water maintained at 45 °C with continuous stirring until completely dissolved and left in the refrigerator for another 24 h to remove air bubbles. KTM and mannitol (5%) have been added to polymer solution. Polyethylene glycol as a plasticizer is added and homogenously mixed with the solutions (Table 1). A specific amount of each of the polymeric solutions was poured in plastic rounded molds of 1.5 cm diameter and then lyophilized over a 30 hr period from 25 °C to − 50 °C after initially being cooled from room temperature, transferred to − 80 °C over a period of 24 hr. KTM wafer was cut into the dimension of 2.5 × 2.5 cm^2 in size in which 10 mg of KTM was included [16]. The best formulated wafers are subjected to different evaluation parameters [19].

2.5 Evaluation Criteria of Wafers

2.5.1 Appearance and Good Appearance

Appearances of wafers were evaluated by visual observation [20].

2.6 Weight Variation

Weight variation was tested in 20 different randomly selected wafers from each batch, and patch thickness was measured at five different randomly selected spots using a micrometer screw gauge [20].

2.6.1 Morphology of Wafers

Surface morphology of the KTM wafer examined by SEM (Scanning Electron Microscope) where samples placed on double-sided adhesive tapes and scanning electron photographs was taken at 35 × magnification [21].

2.6.2 KTM Wafer Disintegration Time Test

Using disintegration apparatus filled with 500 mL phosphate buffer, pH 6.8 at 37 ± 0.5 °C, KTM wafer immersed, and time to break wafer was recorded. [20].

2.6.3 Surface pH Value

The surface pH of the wafers was determined in order to investigate the possibility of any irritation in vivo. The wafers were allowed to swell by keeping them in contact with 1 ml of distilled water for 2 hr at room temperature in specially fabricated glass tubes, and the surface pH was noted by bringing

Table 1 Design of the prepared wafers

Formula	Sodium alginate (%)	Carboxy methylcellulose (%)	Hydroxy propyl methyl cellulose E15 (%)	Ketorolac tromethamine (KT) (mg)	Mannitol (mg)	Propylene glycol (ml)
F1	2%	-	-	60	10	2
F2	6%	-	-	60	10	2
F3	-	2%	-	60	10	2
F4	-	6%	-	60	10	2
F5	-	-	2%	60	10	2
F6	-	-	6%	60	10	2

the electrode in contact with the surface of the wafer, and allowing it to equilibrate for 1 min [20].

2.6.4 Drug Content Loading Capacity

KTM wafer was added to 10 mL of phosphate buffer pH 6.8 and kept aside until wafer dissolves completely, then 2 mL was taken and diluted to 10 mL with phosphate buffer pH 6.8. The solution was analyzed by UV–Visible spectrophotometer at 320 nm [22].

2.7 In Vitro Drug Release Test

The rotating paddle method (USP 23, 2000) was used to study the drug release from the wafers used to assess wafer release profile. A quantity of 500 mL phosphate buffer and pH 6.8 at 37 ± 0.1 °C with 75 rpm containing KTM wafer was prepared. Samples of 10 ml of the dissolution medium were taken and analyzed at different time intervals for 1 hr. The concentration of KTM in each sample was measured spectrophotometrically at wavelengths 320 nm, respectively. [23]

2.7.1 Kinetic Study of KTM Wafer

The in-vitro release study was studied using different kinetic equations models [24].

2.7.2 Chromatographic and Mass Spectrometric Conditions

The mobile phase used was isocratic acetonitrile and ammonium formate (80:20, v:v). The flow rate was 1 mL/min, the run time was 1 min, and the injection volume was 25 μl. The mass spectrometric detection was performed on API-3200 triple quadrupole MS/MS detector (AB Sciex, Toronto, Canada) using multiple reactions monitoring (MRM). The electrospray ionization (ESI) interface was set in the positive ionization mode. The mass spectrometer was equipped with a turbo ion spray interface at 400 °C. The ion spray voltage was set at 5500 V. The common parameters curtain gas, nebulizer gas, and turbo gas were set at 20, 20, and 50 psi, respectively. The collision energy, declustering potential, entrance potential, and collision exit potential were 27, 51, 10, and 14 V, respectively, for KT and for IS 23, 31, 6, and 28 V, respectively. Multiple reactions monitoring (MRM) mode detection of the ions was performed in the monitoring transition of the 256.2 ➔ 105.2 m/z for KT and 349.0 ➔ 263.90 m/z for IS.

2.7.3 Sample Preparation

The standard stock solution of KTM was prepared by dissolving an equivalent weight of 40 mg in 100 mL of acetonitrile, water (50:50, v:v), so that the final concentration is 400 μg/mL. Then, the working solutions were prepared by further dilution with mobile phase. The standard stock solution of IS (200 μg/mL) in acetonitrile, water (50:50, v:v), was prepared. After, the working solution of the IS were prepared by dilution with mobile phase, so that the final concentration is 200 ng/mL.

The plasma calibrators were prepared by spiking 1 mL plasma sample with increasing quantities of standard working solutions, in a centrifuge tube, so that the final concentrations are 1, 2, 20, 100, 400, 800, 1200, 1400, 1800, and 2000 ng/mL. After, all samples were spiked with IS working solution (200 ng/mL). Hence, each of the plasma calibrators was mixed using vortex mixer for 10 s. The quality control samples (QC) were prepared using the same method as calibrators; the lower limit of quantification (LLOQ), low (LQC), medium (MQC), and high (HQC) were 1, 3, 1000, and 1600 ng/mL, respectively.

For extraction, 100 μL of HCL was added separately to each sample. Then, 4 mL tertiary butyl methyl ether was added to the sample. Each sample was vortexed for 30 s, then centrifuged at 4000 rpm for 5 min, and the supernatant was separated and evaporated to dryness using centrifugal vacuum concentrator at 45 °C. The dry residue was then dissolved in the mobile phase and vortexed for 1 min. An aliquot of 25 μL was loaded into LC-MS/MS.

2.7.4 Validation of the Analytical Method

The method was validated to the accepted guidelines of food and drug administration (FDA) (2001) for bioanalytical method validation [25].

2.7.5 Selectivity

It is the ability of the method to quantify an analyte in the presence of different endogenous compounds in the sample. It was investigated by analyzing six different blank plasma samples for interference of endogenous components with the analyte, under the same chromatographic conditions, mentioned earlier. The possible interferences were investigated at the same retention time (tR) and m/z of KTM and IS.

2.7.6 Linearity, Lower Limit of Quantification (LLOQ), and Limit of Detection (LOD)

For linearity, plasma calibration curve was performed using ten calibration standards at levels of 1, 2, 20, 100, 400, 800, 1200, 1400, 1800, and 2000 ng/mL. The plasma calibrators were prepared as mentioned earlier. The calibration curve was constructed in which y was the peak ratio of KTM to IS and x is the concentration of the analyte KTM.

The lower limit of quantification (LLOQ) is the concentration that demonstrates a signal to noise ration higher than or equal 5, with a precision less than 20% coefficient of variation (CV%) and accuracy of 80–120%. As for the limit of detection (LOD), it is the concentration that demonstrates a signal to noise ratio equal to 3.3.

2.7.7 Precision and Accuracy

The precision of the method is how close the results obtained from the analysis to each other, while the accuracy of the method is how close the results to the true value.

In order to evaluate the intra-day precision and accuracy, seven samples of each QC samples (3, 1000, and 1600 ng/mL KTM) were analyzed within the same day and analyzed on 3 different days to evaluate the inter-day precision and accuracy. The intra- and inter-day precision was expressed by percent coefficient variation (% CV), while the % accuracy was determined by % recovery = [(mean found concentration)/(nominal concentration)] × 100.

2.7.8 Matrix Effect and Extraction Recovery

Blank rat plasma samples from six different sources were extracted. Each was spiked with KTM at QCL level and IS at 200 ng/mL. In order to evaluate the matrix effect on ionization of KT and IS, the peak area of KTM and IS in the post-extracted samples is compared with their standard working solution at the same concentration.

To evaluate the extraction recovery, the peak areas of the QC samples (QCL, QCM and QCH) and IS in the pre-extraction samples are compared with their peak areas in the post-extraction samples. It is worth mentioning that the pre-extraction samples were prepared by spiking plasma with sample and then they were extracted, while the post-extraction samples were prepared by spiking plasma with sample at the end of the extraction process [26].

2.7.9 Dilution Integrity

To ensure that the dilution of the sample with the biological matrices does not affect the accuracy of the method, dilution integrity test took place. Plasma was spiked with concentration higher than upper limit of quantification (ULOQ) 3000 ng/mL KT, then it was diluted with plasma so that the final concentration is 1000 ng/mL. The effect of dilution was evaluated by average % recovery calculation of the six replicates.

2.7.10 Stability Study of the Analyte

The stability samples were two levels of the QC samples (QCL and QCH). First, three sets of QCL and QCH samples were prepared and analyzed for each one of the stability studies investigated in this work. In order to evaluate the stability in the matrix, the mean recovery obtained from the stored stability samples is compared with that obtained using freshly prepared samples, at the same concentration levels. The % deviation should not exceed 15% [25, 26].

2.7.11 Short-Term Stability

The stability samples were left at room temperature for 12 hr before analysis. This time is expected to be the maximum time expected for routine sample preparation.

2.7.12 Long-Term Stability

The stability samples were stored at − 70 °C for 119 days. The storage time exceeded the time between the date of first sample collection and the date of last sample analysis.

2.7.13 Post-Preparative Stability

The stability samples were prepared and kept in the autosampler at 25 °C for 12 hr. This time is expected to be the maximum time of storage of the sample in the autosampler.

2.7.14 Freeze and Thaw Stability

It was evaluated by subjecting the stability samples to three freeze (− 70 °C) and thaw (room temperature) cycles. At each cycle, the stability samples were frozen at − 70 °C for 24 hr, then thawed at room temperature for 2 hr.

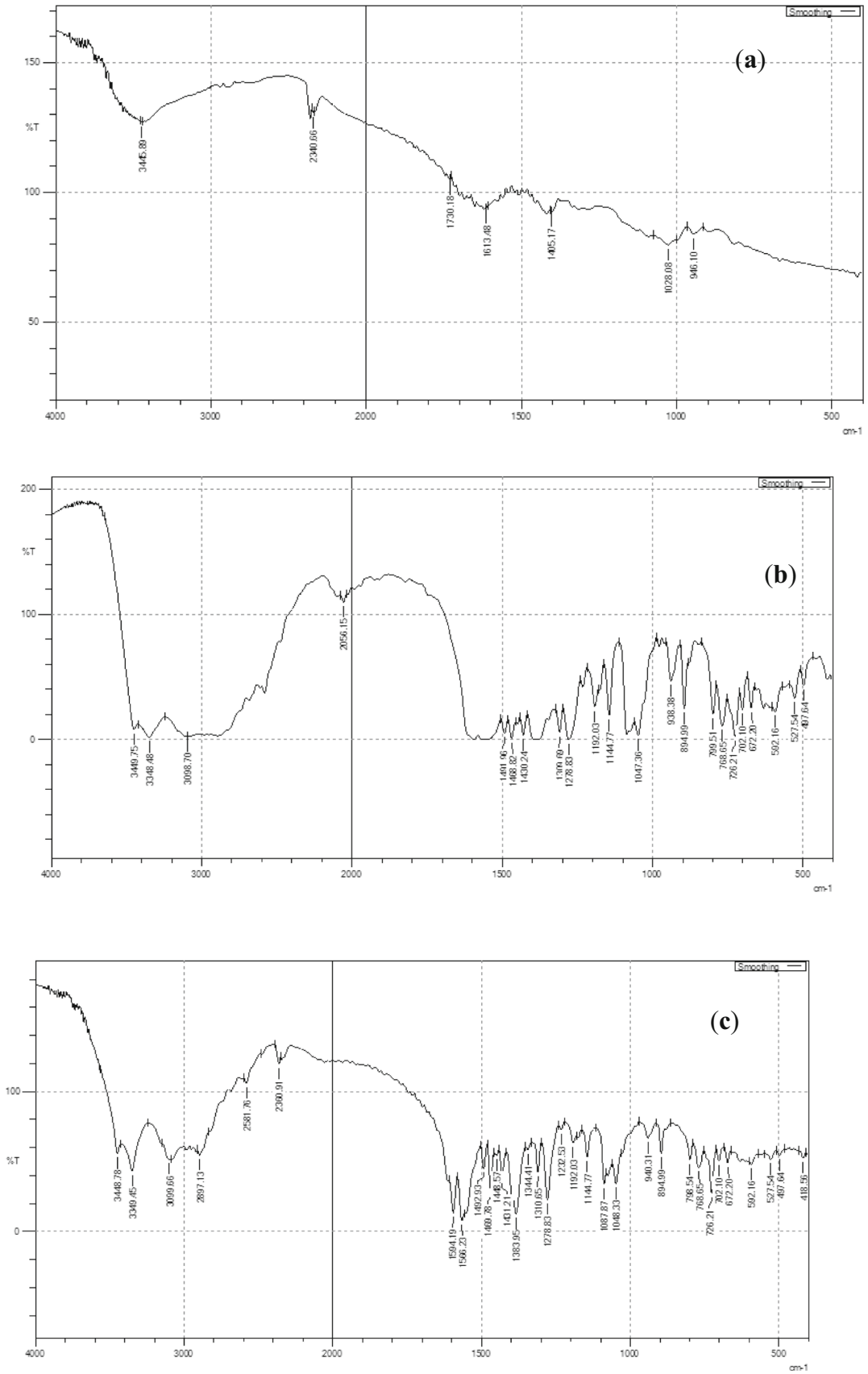

Fig. 2 IR spectrum for ketorolac **a**, sodium alginate **b**, and ketorolac and sodium alginate **c**

Table 2 Physicochemical properties of ketorolac tromethamine wafers

• Formula code	• Weight variation (g)	• Drug content (%)	• Surface pH	• Disintegration time test (sec)
• F1	• 0.004 ± 0.02	• 96.5 ± 0.02	• 6.6 ± 0.02	• 60
• F2	• 0.005 ± 0.01	• 102.5 ± 0.02	• 6.6 ± 0.02	• 85
• F3	• 0.006 ± 0.05	• 95.89 ± 0.02	• 7 ± 0.02	• 167
• F4	• 0.007 ± 0.02	• 93.966 ± 0.02	• 6.8 ± 0.02	• 200
• F5	• 0.004 ± 0.04	• 95 ± 0.02	• 7 ± 0.02	• 245
• F6	• 0.004 ± 0.04	• 95 ± 0.02	• 7 ± 0.02	• 256

2.8 Pharmacokinetic Study

2.8.1 Study Design

The present study aimed at comparing the pharmacokinetic parameters of KTM best wafer with commercially available KTM tables. Eighteen male Wistar rats (8 weeks old, weighing 150–200 g) were used. Animals were obtained from the National Scientific Research Center (Giza, Egypt). They were fed a standard pellet chow (El-Nasr Chemical Company, Cairo, Egypt) and had free access to water. All animals were housed in plastic cages with no more than three rats per cage and were kept in a conditioned atmosphere at 25 °C. The animals were randomly divided into the following three groups: negative control group which received saline by oral administration, standard group which received 1 ml of suspension containing 10 mg of the commercially available KTM tablets orally, and test group in which 2 cm^2 wafers (containing an equivalent of 10 mg KTM) were cut into four pieces and placed between the cheeks of the oral buccal cavity. Blood samples (250 μl) were then collected from the tail vein in heparinized tubes at 0, 15, 30, 60, and 120 min. Plasma was then separated by centrifugation at 4000 rpm for 15 min [27].

2.8.2 Ethics Statement

All experiments were performed in accordance with relevant guidelines and regulations. The study and all the experimental protocols were conducted after the approval of the ethics committee at October University for Modern Sciences and Arts (MSA).

2.9 Pharmacokinetic and Statistical Analysis for Wafer

Peak plasma concentration (C_{Pmax}), the time to reach peak plasma levels (T_{max}), area under the curve (AUC), and relative bioavailability were calculated using Kinetica software (Kineitca 2000 version 3.0, InnaPhase Corporation, 2000). [28].

3 Results and Discussion

3.1 Fourier Transform Infrared Spectroscopy Studies

It was performed to detect physical interaction between the drug and sodium alginate. IR spectrum of pure KTM shows peak at 1047.59 cm^{-1} due to -OH bending confirms presence of alcoholic group, peaks at 3350.01 cm^{-1} due to N-H and NH_2 stretching, peak at 1383.19 cm^{-1} is due to C–N vibrations, peaks at 702.09 cm^{-1}, 725.54 cm^{-1}, 771.71 cm^{-1}, and 798.11 cm^{-1} confirm C–H bending (Aromatic), peaks at 1469.43 cm^{-1} and 1430.88 cm^{-1} due to C=C aromatic and aliphatic stretching, thus confirms structure of KTM, as presented in (Fig. 2a). Spectrum of sodium alginate (Fig. 2b) showed stretching vibrations of O–H bonds of alginate appeared in the range of 3000–3600 cm^{-1} important absorption bands regarding hydroxyl, ether, and carboxylic functional groups. The spectra showed no incompatibility between the sodium alginate; polymer of best wafer formed and KTM drug are shown in (Fig. 2c).

3.2 Characterization of KTM Lyophilized Wafers

Many trails to formulate the KTM wafers from different concentrations of HPMC E15 and sodium alginate. Wafers with 2% w/v concentration for both polymers were very thin, brittle, and easily broken, while wafers with 6% w/v concentration from both polymers were clear, transparent, and have good mechanical properties. Wafers present with uniform weight between 96.6 and 107.7 mg with SD values 0.4–0.8. Surface pH within a range of 6.6–7 was compatible with mucosa pH. All fast melt wafers contain uniform quantity of the drug ranging from 93.967 to 102.5%. Disintegration time was almost fast, ranging from 1 to 4 min; results were shown in Table 2.

3.3 Morphology of the Prepared Wafers Using Scanning Electron Microscopy

The SEM was used to detect shape of wafers. All different polymers show network of sponge-like structure with porous morphology (Fig. 3).

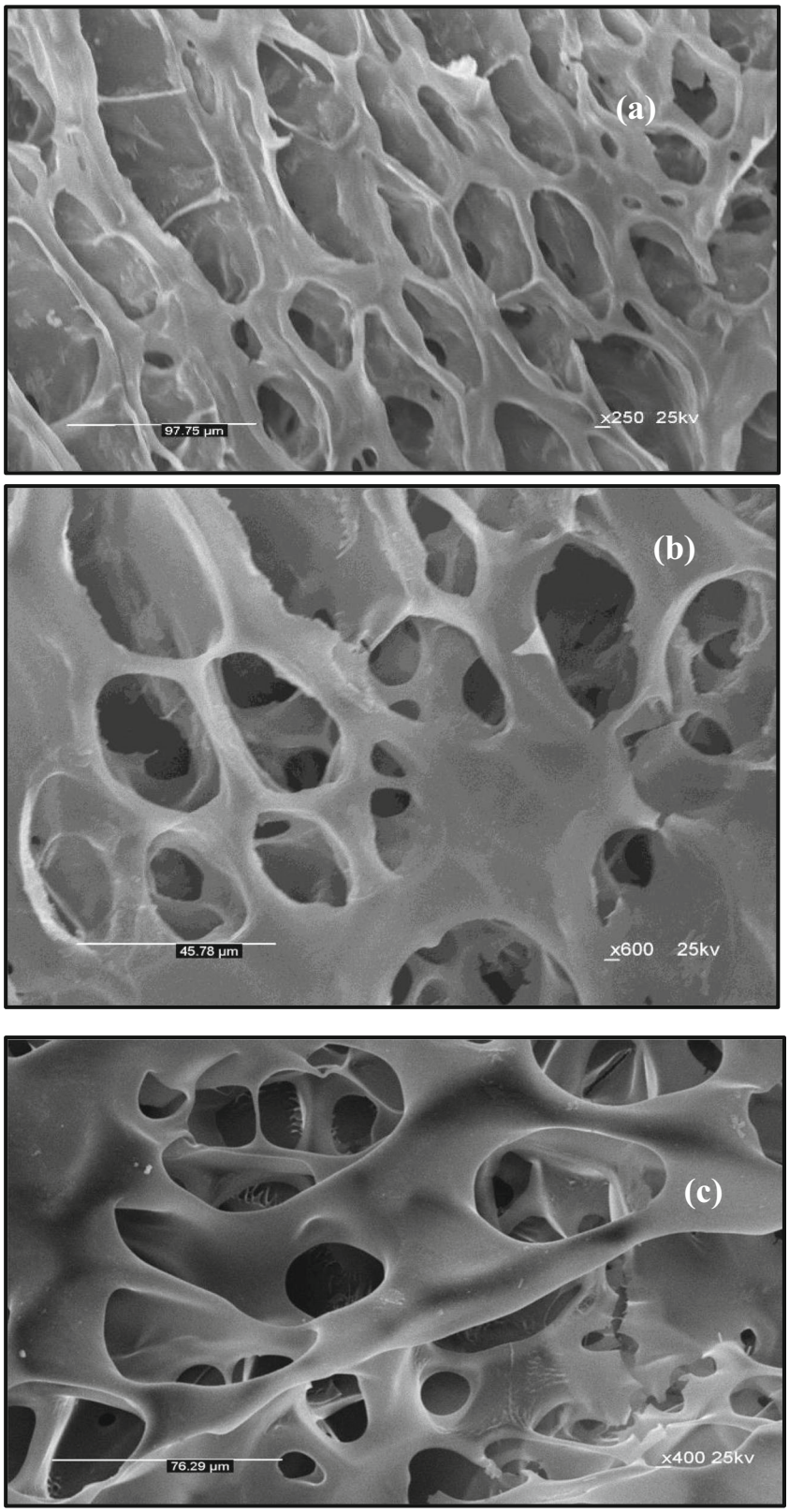

Fig. 3 SEM of the prepared wafers: sodium alginate wafer **a**, sodium carboxy methyl cellulose **b**, and hydroxypropyl methyl cellulose wafer **c**

3.3.1 In Vitro Release Test for KTM Fast Melt Wafer

The release of drug from the dosage form plays important roles in buccal drug delivery and in determining the therapeutic effect of the medication. Upon application of a polymeric wafer, drug releases from the dosage form to the oral cavity, dissolved in the salivary film, partitions into the superficial layers of epithelium, diffuses through

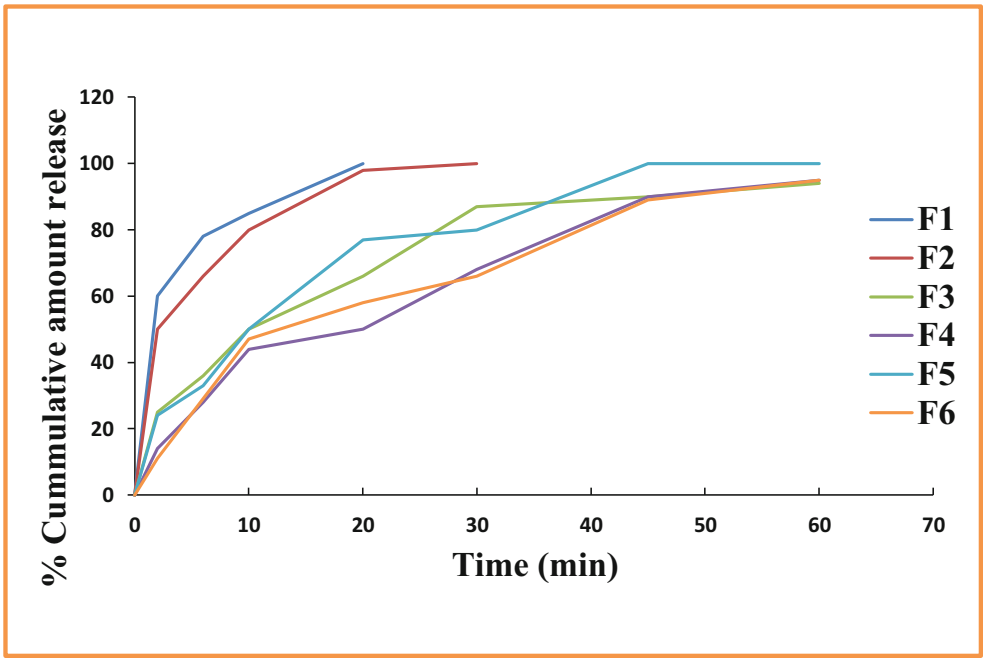

Fig. 4 The % cumulative amount released of ketorolac tromethamine from wafers

the epithelial layers, and then partitions into and is transported away by the blood [29]. The in vitro drug release of the drug from buccal medicated films (films F1–F6) was studied at 37 ± 0.5 °C using phosphate buffer (pH 6.8) as the release medium. The percent release of the drug as function of time is presented in Fig. 4. It is found that the release of KT was fast from all polymers as they are hydrophilic polymers and upon hydration; KT release would be expected to occur through channels formed due to the dissolving of polymer and swelling mechanisms occurs, and the drug release is generally facilitated by the porous network. The porous hollow structure with increased surface area of the dispersed KTM increase the dissolution rate. As shown in (Fig. 4) and Table 3, the total cumulative percent KTM rapid melt wafer release in 1 hr from all formulae ranged from 94 to 100%. Formulae F1 and F2 with sodium alginate show rapid release due to the high swelling characterization of alginate porous structure; while F3 and F4 wafers with carboxymethyl cellulose show slow release as the CMC, which itself has a stable network structure thus increases the tensile strength of the composite wafer being more hard structure; F5 and F6 formulae with HPMC show also fast release rate due to high saliva uptake but less than sodium alginate. F2 wafer with sodium alginate 6% showed faster drug release in less than half an hour. Sodium alginate is considered the best polymer to release drug within a very short time. Charged polymers such as sodium alginate demonstrate higher adhesion than non-ionic polymers (e.g., HPMC), because of their ability to form a strong electrostatic interaction with the charged surface of mucin [30].

Table 3 The % cumulative amount released from wafers

Time (min)	% cumulative amount released from formulae					
	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
2	60	50	25	14	24	11
6	78	66	36	28	33	29
10	85	80	50	44	50	47
20	100	98	66	50	77	58
30		100	87	68	80	66
45			90	90	100	89
60			94	95	100	95

3.4 Kinetic Study of Fast Melt KTM Wafer

The obtained values of correlation coefficient are depicted in Table 4. The order of drug release from all the formulations was studied, and it appears to be as it follows second order. Selection of the kinetic release of F2 best formula indicates the releases of KTM from the patches were diffusion rate controlled.

3.4.1 Optimization of the Chromatographic Methods

A new LC-MS/MS was developed for the identification and quantification of KT in plasma. Hence, the proposed method

Table 4 Drug release kinetics studies of ketorolac tromethamine (KT) buccal tablets

	Zero order (R^2)	First order (R^2)	Second (R^2)	Diffusion (R^2)	Hixon (R^2)	Baker (R^2)	Chosen (R^2)
F1	0.666404	− 0.61874	0.927111	0.836667	0.888023	0.928911	0.92891053
F2	0.71822	− 0.62464	0.920978	0.880902	0.847755	0.863055	0.920978
F3	0.71822	0.900208	0.920978	0.880902	0.847755	0.863055	0.920978
F4	0.957475	− 0.26207	0.921768	0.993337	0.991253	0.979639	0.993337
F5	0.71822	0.912905	0.920978	0.880902	0.847755	0.863055	0.920978
F6	0.71822	0.944135	0.920978	0.880902	0.847755	0.863055	0.944135

was applied in the pharmacokinetic study. In order to reach the optimum condition, different factors have been studied. The most intensive product ion was observed at m/z 256.2 for KT and m/z 349.0 for the IS. Therefore, the precursor-to-product ion transitions m/z 256.2 → 105.2 for KT and m/z 349.0 → 263.9 for IS in the selected reaction monitoring mode were used for the quantitation of KT and IS. Different mobile phase combinations, consisting of acetonitrile, methanol, formic acid in water, and ammonium formate were tested. The best parameters of the mobile phase were found with acetonitrile: ammonium formate (80:20, v/v). Under the optimum condition, the tR of KT and IS was 0.477 and 0.489 min, respectively; as shown in (Fig. 5). For extraction, tertiary butyl methyl ether was used because of its high extraction efficiency and low interference.

4 Validation of the Analytical Method

4.1 Selectivity

The selectivity of the method was confirmed by the absence of any interferences peak, from either plasma components, at the tR or m/z of KT or IS. The specificity of the method also demonstrated by the absence of interfering peaks of the commonly used drugs in the subject population including acetaminophen, acetylsalicylic acid, ibuprofen, caffeine, nicotine, and ascorbic acid has been investigated and no interfering peaks detected.

4.1.1 Linearity, Lower Limit of Quantification (LLOQ), and Limit of Detection (LOD)

The method was linear over the concentration range 1–2000 ng/mL. The regression equation was $Y = 0.0033x - 0.0338$, in which y is the peak area ratio (KT to IS) and x is KT concentrations. The linearity of the method was evaluated by the good correlation coefficient value 0.9940.

As the LLOQ is defined as the lowest concentration that can be measured with acceptable accuracy and precision, it was found to be 1 ng/mL in rat plasma with a precision of 5.07% and accuracy of 112.45%. The detection limit (LOD) was regarded as the lowest amount of the analyte, which can be detected but not necessarily quantitated; it was found to be 0.26 ng/mL.

4.2 Precision and Accuracy

The intra-day precision (%CV) and accuracy of the method ranged from 4.485 to 5.791% and 103.036 to 106.714%, respectively. As for the inter-day precision (%CV) and accuracy of the method, it ranged from 5.56 to 8.291% and 100.45 to 104.429%, respectively. Accordingly, for intra- and inter-day precision the % CV was less than 8.291% and for intra- and inter-day accuracy, the results were also in the acceptable range (± 15%). The data is presented in Table 5.

4.3 Matrix Effect and Extraction Recovery

The analyte ionization in the ion source is evaluated by the matrix effect. It is represented by the ratio of the peak area of the spiked post-extracted samples of KT and IS to the peak area of their standard working solutions at the same concentration.

The ratio ranged from 0.9190 to 1.0373, with %CV of 4.865%, which indicated that there is no significant effect of the matrix on the ionization of KT or the IS.

For extraction recovery evaluation, the average % recoveries of the six replicates were 76.31 ± 3.10, 94.71 ± 2.47, and 82.42% ± 2 for QCL, QCM, and QCH, respectively.

4.4 Dilution Integrity

The average % recovery was 98.73 ± 2.27, which indicates that the dilution has no effect on the accuracy of the method.

4.5 Stability Assessment

From the data presented in Table 6, for all the stability studies investigated in this work, it can be concluded that

there are no significant differences between the fresh samples and the stability stored samples. This can be explained that there is no significant degradation of KT or IS when stored under the previously mentioned conditions.

4.6 Pharmacokinetic Parameters of KTM in Rats

Pharmacokinetics parameters were investigated of KTM following oral administration of 10 mg wafer (F2) and 10 mg of the marketed product as shown in Table 7. The mean Cp_{max}

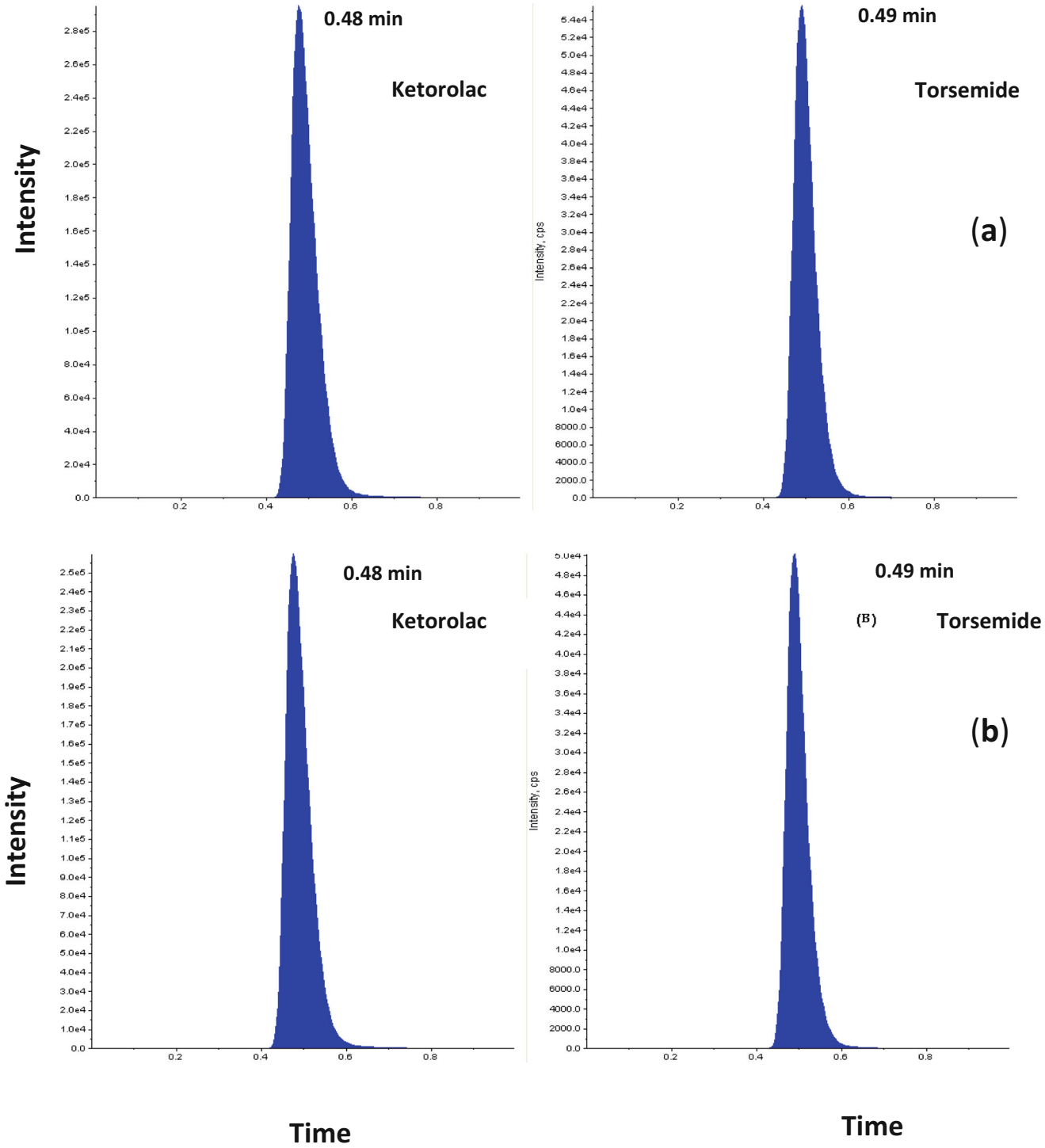

Fig. 5 Representative chromatograms for a standard mixture containing ketorolac (1600 ng/mL) and torsemide (IS) (200 ng/mL) in the mobile phase **a** and in plasma **b**

Table 5 Intra- and inter-day precision and accuracy of ketorolac tromethamine (KT) in rat plasma

QC samples (ng/mL)	Intra-day ($n = 7$)		Inter-day ($n = 7$/day) for 3 days	
	Precision (%CV)	Accuracy (%recovery)	Precision (%CV)	Accuracy (%recovery)
3	5.647	105.619	6.996	104.429
1000	4.485	106.714	8.291	101.552
1600	5.791	103.036	5.56	100.45

Table 7 Pharmacokinetic parameters of ketorolac tromethamine (KT) in rats

Parameters	F2 wafer	Conventional tablet
C max (ng/ml)	1073 ± 23.56	2135.47 ± 13.83
T max (h)	1.00 ± 0.04	3 ± 0.07
AUC0−∞(ng-h/ml)	2087 ± 71.58	3981.189 ± 62.34
MRT (h)	1.13 ± 0.02	3.86 ± 0.02

Relative bioavailability, AUC of F2 wafer/AUC of conventional tab = 0.523

values for F2 and the marketed product were 2135.47 ± 13.83 ng/mL and 1073 ± 23.56, respectively. The median values of T_{max} were 1 and 3 hr, respectively. The increase in the extent of absorption of KTM from the prepared wafer (F2) is also distinguished from $AUC_{0-\infty}$ values for both treatments. The calculated $AUC_{0-\infty}$ values were 2087 ± 71.58 and 3981 ± 62.34 ng h/mL for F2 and marketed product correspondingly. Compared with the commercially available tablets, the relative bioavailability was found to be 0.52. A new application of KTM wafer is the developed ketorolac/lidocaine polymeric wafer to reduce pain together with enhancing wound healing following gingivectomy [10]. A buccal KTM film was clinically evaluated widely by post-oral surgery pain model [31]; this ensures that buccal wafer is a new technology that enhances the dissolution and absorption of drugs. KTM wafer approaches high plasma concentration in a very short time reducing many side effects and increase patient compliance.

5 Conclusions

In this work, six wafer formulations were developed. On the basis of characterization and in vitro release of all six wafer formulations, formula F2 contains 6% sodium alginate as the formulation of choice for the pharmacokinetic study. It shows disintegration time in 85 s, with surface pH 6.6, drug content with 102%, and high dissolution rate in 20 min. The mean C_{pmax} values for F2 and the marketed product were 1073 ± 23.56 and 2135.47 ± 13.83 ng/mL, respectively. The median values of T_{max} were 1 and 3 hr, respectively. The calculated AUC0 − ∞values were 2087 ± 71.58 and 3981 ± 62.34 ng h/mL for F2 and marketed product, correspondingly. Accordingly, the relative bioavailability was found to be 0.52; this is due to that the wafer rapidly dissolves in the buccal cavity leading to absorption of the active ingredient via the oral mucosa into the bloodstream, bypassing the liver's first pass effect hence improving bioavailability. A newly LC-MS/MS was developed and validated to perform the pharmacokinetic and the bioavailability study. The method was linear 1–2000 ng/mL, with 0.26 ng/mL detection limit.

Table 6 Stability data of ketorolac tromethamine (KT) under different conditions

QC samples	Post-preparative stability (%deviation)	Freeze and thaw stability (%deviation)	Short-term stability (%deviation)	Long-term stability (%deviation)
QCL	− 2.08	− 3.35	− 5.73	7.79
QCH	− 8.46	− 8.45	0.22	− 3.77

%deviation = ((stability sample−fresh sample)/fresh sample) × 100

Compliance with Ethical Standards

Conflict of Interests The authors declare no competing interests.

Informed Consent None.

Research Involving Humans and Animals Statement All experiments were performed on rats in accordance with relevant guidelines and regulations. The study and all the experimental protocols were conducted after the approval of the ethics committee at October University for Modern Sciences and Arts (MSA).

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