ORIGINAL RESEARCH

Preparation, Characterization, and *In-Vitro* Evaluation of Tenoxicam-Paracetamol Cocrystal

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ABSTRACT

Crystallization is a promising approach in enhancing drug solubility and dissolution. The objective of this study was to prepare and evaluate tenoxicam co-crystals with paracetamol as a co-former. Co-crystals of tenoxicam-paracetamol were prepared by solvent evaporation technique and grinding method; three ratios of the drug to conformer were used (1:1, 1:2, and 1:4). The tenoxicam-paracetamol co-crystal was characterized by determining the melting point Fourier transform infrared spectroscopy, powder X-ray diffraction (XRD), and Scanning electron microscopy. The co-crystals were evaluated for the drug solubility and dissolution, the content of tenoxicam within the co-crystals was also determined. The formula with the ratio (1:2) tenoxicam to paracetamol that was prepared by solvent evaporation technique had a different melting point than that of the pure drug and of the conformer, the Fourier transform infrared spectroscopy analysis indicated the formation of hydrogen bond and the powder X-ray diffraction pattern of the co-crystal showed a unique Powder XRD pattern distinguishable from that of tenoxicam and paracetamol. The formula with the ratio (1:2) tenoxicam to paracetamol that was prepared by solvent evaporation technique showed the best enhancement in drug solubility and dissolution compared to the other formulae. In conclusion, solvent evaporation technique and the ratio of (1:2) tenoxicam to paracetamol are more efficient in enhancing drug solubility and dissolution than the grinding method and other ratios used, respectively.

Keywords Co-crystals, Tenoxicam, Solvent evaporation, Grinding, solubility.

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INTRODUCTION

In the pharmaceutical industry, many research-based pharmaceutical companies have invested huge amounts of money in developing new chemical entities and about 40% of these entities have poor water solubility. Low aqueous solubility is one of the major problems that can confront pharmaceutical scientists as they formulate new chemical entities because any drug should be in an aqueous solution to be absorbed at its site of absorption. According to the biopharmaceutical classification system (BCS), dissolution is a rate-limiting step in oral absorption of active ingredients that belong to BCS class II and IV that have low solubility.[1,2]

Many approaches have been proposed to improve drug solubility and dissolution; one of these techniques is cocrystallization, which has been investigated thoroughly in recent years. Co-crystals are defined by European Medicine Agency (EMA) as *'homogenous (single-phase) crystalline structures made up of two or more components in a definite stoichiometric ratio where the arrangement in the crystal lattice is not based on ionic bonds (as with salts),'* and another definition is provided by United States Food and Drug Administration (USFDA) which states that *'crystalline materials composed of two or more molecules in the same crystal lattice'*. The drug and co-former molecules are stabilized by non-covalent interactions as hydrogen bonding within the crystal lattice. This technique has a great impact in tailoring many drug physicochemical properties as solubility, processability and thermal stability.[3-5]

Tenoxicam (TX) is a non-steroidal anti-inflammatory drug (NSAID); it acts by the inhibition of cyclooxygenase enzyme which is involved in prostaglandin biosynthesis. It is used in the symptomatic pain relief and treatment of many inflammatory diseases as arthritis, musculoskeletal disorders, and toothaches. Tenoxicam has an elimination half-life range of 60-75 h, and it has two pKa values of (1.3) and (5.3). It is administered as a single oral daily dose of 20 mg. [6,7]

Tenoxicam is very slightly water-soluble drug and belongs to BCS class II drugs, thus dissolution may represent a rate-limiting step in its absorption process.[8,9]

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The aim of this study is to enhance TX solubility and dissolution rate by preparing a new co-crystal of TX and paracetamol as a co-former.

MATERIALS AND METHODS

Materials

Tenoxicam and paracetamol were both obtained from Middle East laboratories Co. limited (Iraq), while the other materials and solvents are of reagent or analytical grade.

Methods

Preparation of TX-paracetamol Co-crystals

Solvent evaporation technique

This technique prepared three formulae, TX and paracetamol at ratios of 1:1, 1:2, and 1:4, as shown in Table 1, were dissolved in methanol using a magnetic stirrer; the obtained solution was placed in a water bath and allowed to evaporate. The dry powder was obtained by scratching the beaker and the resultant dry powder was kept for characteriztion.[10]

Grinding technique

Three formulae were prepared using this technique; TX and paracetamol at ratios 1:1, 1:2, and 1:4, as shown in Table 1, were grinded finely using mortar and pestle. The powder mixture was scratched from the mortar and then kept for characterization.[11]

Characterization of Tenoxicam and TX-paracetamol co-crystals

Melting Point Determination

The melting point of the drug TX, paracetamol and the selected formula was measured using melting point apparatus (Barnstead electrothermal 9100, USA).

Fourier Transform Infrared Spectroscopy (FTIR)

Tenoxicam, paracetamol, and the selected formula of TX-paracetamol co-crystal FTIR spectra were obtained using pressed disk technique. A small amount of the sample was ground with potassium bromide powder then the mixture was pressed into a disk. The infrared spectra were obtained using an infrared spectrophotometer (Thermo scientific iS^{TM}5 FT-IR, USA) in a wave number range of (4000–400 cm^{-1}).[12]

Powder X-ray Diffraction (XRD)

The powder X-ray diffraction analysis of TX, paracetamol, and the selected formula of TX-paracetamol co-crystal was done at room temperature using (XRD-6000 Shimadzu, Japan). The measurement conditions were set as following: the x-ray target was Cu, the radiation wavelength λ = 1.54 Å, the voltage and current were set at 40 kV and 30 mA, respectively. The analysis results were measured at 2 Θ axis with angular range of 5-80°, and at a rate of 5°/min.[10]

Table 1: The technique and ratio of each formula

Formula	*TX: Paracetamol ratio w/w*	*Cocrystallization technique*
F1	1:1	Evaporation
F2	1:2	Evaporation
F3	1:4	Evaporation
F4	1:1	Grinding
F5	1:2	Grinding
F6	1:4	Grinding

Scanning Electron Microscopy (SEM)

The morphology of TX, paracetamol and the selected formula crystals were examined using SEM (Vega III, Czech Republic) at magnification 5Kx. The sample was placed on an aluminum stub; a coater sputter used to coat the sample with 10 nm gold layer and the accelerating voltage was set at 10 kV.[5]

Evaluation of Tenoxicam and TX-paracetamol Co-crystals

Solubility Determination

The solubility of TX and the TX-paracetamol co-crystal powder were determined in both distilled water and phosphate buffer pH 6.8. An excess amount of TX and the co-crystal powder were mixed with 10 mL of both solvents and placed on a magnetic stirrer for 48 hours at 37ºC. The solution was filtered using a 0.45 µ syringe filter, and the filtrate then was analyzed using UV spectrophotometer at TX λ_{max} at 369 nm.[12]

Determination of TX Content in the Co-crystals

The content% of TX in the co-crystal powder for each formula was measured by dissolving a sample powder equivalent to 20 mg of TX in up to 100 mL of methanol. The solution was diluted and analyzed using UV spectrophotometer at 369 nm.[13]

Dissolution Test

This test was done using dissolution apparatus II USP; the dissolution vessel was filled with 900 ml of phosphate buffer pH 6.8 and its temperature was kept at 37 ± 0.5°C and the paddle rotation was set at 50 rpm. A powder sample (equivalent to 20 mg of TX) of the drug and all the formulae were tested, 5ml samples were withdrawn with a syringe at specified time intervals (1, 2, 3, 4, 5, 10, 20, 30, 45, and 60 minutes) and a fresh medium replaced them. Each sample was filtered with 0.45 µ syringe filter and analyzed using UV spectrophotometer at TX λ_{max}. Then the cumulative drug release% of the pure powder and of all the formulae were calculated and compared.[5]

Statistical Analysis

The experimental evaluation data were conducted in triplicates and expressed as means and their standard deviations (mean ± SD). T-test was used to analyze the results of the study and assess the statistically significant differences in comparing means; p-values considered: significant at a level of ($p \leq 0.05$) and not significant at a level of ($p > 0.05$). Similarity factor (f_2) was also used to compare the formulae release profiles; when two curves are identical (f2 = 100), the FDA considers two release curves to be similar when f2 ≥ 50. Equation14 calculates this factor:

$$f_2 = 50 \times \log\left\{\left[1 + \frac{1}{n}\sum_{t=1}^{n}(R_t - T_t)^2\right]^{-0.5} \times 100\right\}$$

RESULTS AND DISCUSSION

Characterization of Tenoxicam and TX-paracetamol Co-crystals

Melting Point Determination

Tenoxicam powder was completely melted at 212ºC and this is consistent with the reported results in references[15], and paracetamol powder was completely melted at 168.9ºC this result is in agreement with the references. [16] The melting point of the selected formula F2 was found to be 191.5ºC.

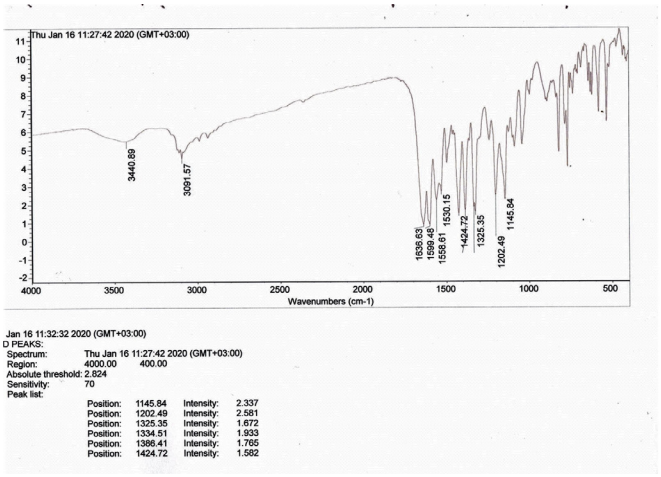

Figure 1: FT-IR spectrum of TX

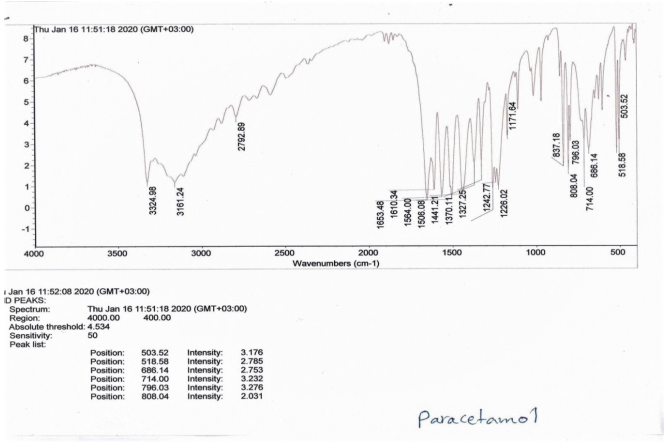

Figure 2: FT-IR spectrum of paracetamol

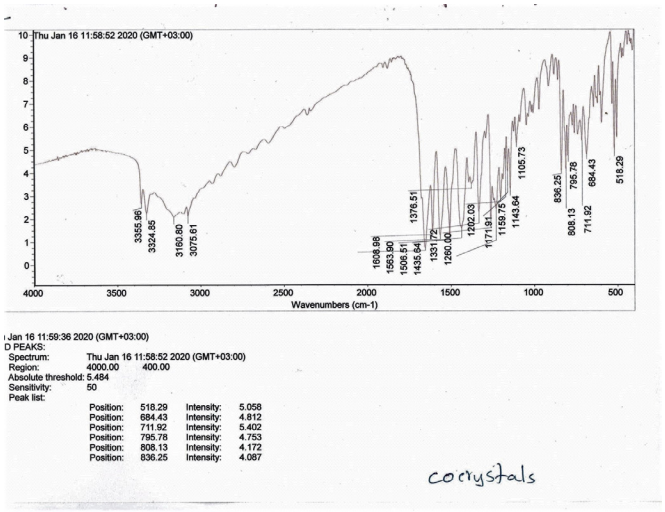

Figure 3: FT-IR spectrum of the selected formula F2

Fourier Transform Infrared Spectroscopy (FTIR)

Pure TX FTIR spectrum showed sharp characteristic peaks of TX at 3440, 3091, corresponding to NH and OH groups, and 1636 corresponding to the carbonyl amide group as shown in Figure 1. The spectrum of paracetamol shows one peak at 3324 due to NH group and peak at 1653 of carbonyl amide group in Figure 2. All these TX and paracetamol peaks appeared in the spectrum of the selected formula F2 in Figure 3 at the same wave range indicating no interaction or structural modification between TX and paracetamol. The shifting of the TX NH group's stretching peak to 3355 indicates the formation of hydrogen bonds in the selected formula F2 co-crystals.

Powder X-ray Diffraction (XRD)

The formation of TX-paracetamol co-crystal is confirmed by X-ray diffraction of each drug alone and of the selected formula F2. From the XRD diagrams which is used to identify the crystal form of drugs and to detect any change or modification in drug crystal and formation of co-crystals, the selected formula F2 shows a unique XRD pattern distinguishable from that of TX and paracetamol as shown in Figures 4, 5 and 6 of paracetamol, TX and F2 respectively.

Scanning Electron Microscopy (SEM)

The SEM images of TX powder in Figures 4c and 5c show the absence of crystalline structure, which indicates its amorphous nature. Whereas SEM images of paracetamol in Figures 4b

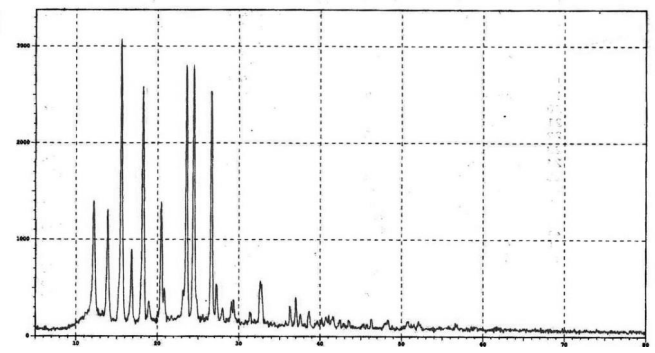

Figure 4: The XRD of paracetamo

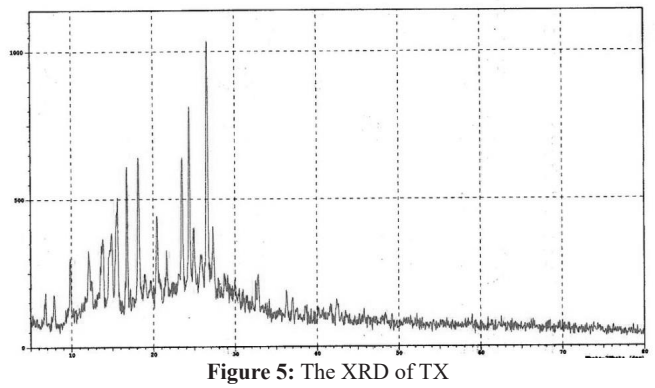

Figure 5: The XRD of TX

and 5b show large crystals and the SEM images of the selected formula F2 in Figures 4a and 5a show aggregates of crystals.

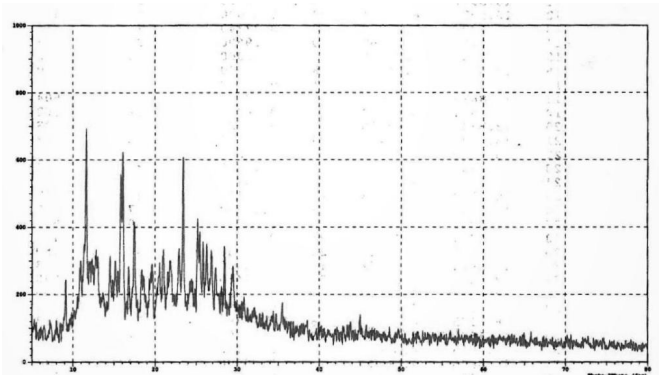

Figure 6: The XRD of the selected formula F2

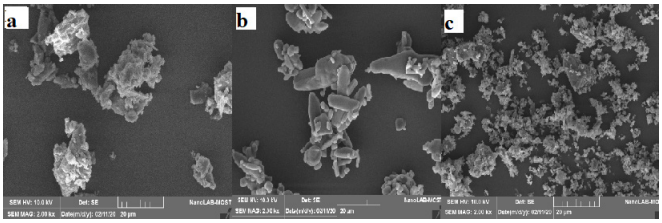

Figure 7: SEM images at magnification 2Kx of a) the selected formula F2 b) paracetamol powder c) TX powder

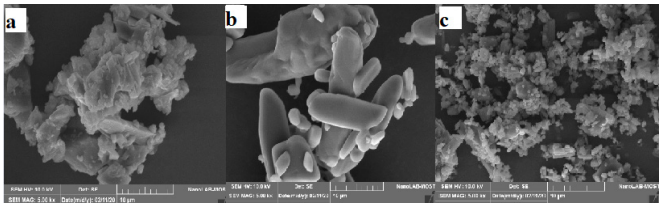

Figure 8: SEM images at magnification 5Kx of a) the selected formula F2 b) paracetamol powder c) TX powder

Table 2: Solubility of TX and the co-crystals in distilled water

Formula	*Solubility (mg/mL) ± SD**
TX	0.060 ± 0.001
F1	0.176 ± 0.002
F2	0.331 ± 0.016
F3	0.090 ± 0.007
F4	0.075 ± 0.002
F5	0.083 ± 0.002
F6	0.063 ± 0.005

*SD: standard deviation

Table 3: Solubility of TX and the co-crystals in phosphate buffer pH 6.8

Formula	*Solubility (mg/ml) ± SD**
TX	0.787 ± 0.003
F1	0.967 ± 0.054
F2	1.000 ± 0.050
F3	0.720 ± 0.046
F4	0.955 ± 0.072
F5	0.685 ± 0.081
F6	0.610 ± 0.007

*SD**:** standard deviation

Evaluation of Tenoxicam and TX-paracetamol co-crystals

Solubility Determination

The saturated solubility studies of TX-paracetamol co-crystal powder showed that all the formulae had a significant increase in water solubility compared to the pure TX with p-values less than 0.05 except for F6 there was no significant change in solubility; the F2 co-crystals is 5.5 folds more water-soluble than pure TX as shown in Table 2.

While the saturated solubility of TX-paracetamol co-crystal powder in phosphate buffer pH 6.8 showed a significant increase for formulae F1 and F2 compared to the pure TX with p-values 0.0281 and 0.0175 respectively, the F1 and F2 co-crystals are 1.2 and 1.3 folds more soluble in phosphate buffer pH 6.8 than pure TX. On the other hand, F6 co-crystals showed a significant decrease in the phosphate buffer solubility compared to the pure drug with a p-value < 0.0001. The rest of the formulae showed no significant change in their phosphate buffer pH 6.8 solubility as shown in Table 3.

Determination of TX content in the co-crystals

The TX content% within the co-crystals are in the range from 89.55 ± 0.20 to 111.75 ± 0.90 as shown in Table 4.

Dissolution test

The dissolution curves of pure TX and the six formulae of TX-paracetamol crystals in phosphate buffer pH 6.8 are shown in Figure 9.

From the drug release profile graph and its statistical analysis data shown in Table 5, the co-crystals in all formulae have significant improvement in the dissolution rate of TX compared to the pure drug, except for F6 that has a similarity factor > 50 which indicates that the pure TX and F6 co-crystals have similar drug release profile and no significant improvement in the dissolution rate.

On the other hand, the co-crystals of F2, which has the lowest similarity factor of 23.55 and complete release of TX

Table 4: The content% of TX in co-crystal powder

Formula	*Content%± SD**
F1	89.55 ± 0.20
F2	103.48 ± 0.30
F3	111.75 ± 0.90
F4	105.24 ± 023
F5	105.57 ± 0.20
F6	99.25 ± 0.11

*SD: standard deviation

Table 5: The similarity factor of drug release from all formulae compared to the pure TX

Formula	f_2 *(%)*
F1	28.67
F2	23.55
F3	33.94
F4	37.71
F5	42.56
F6	51.69

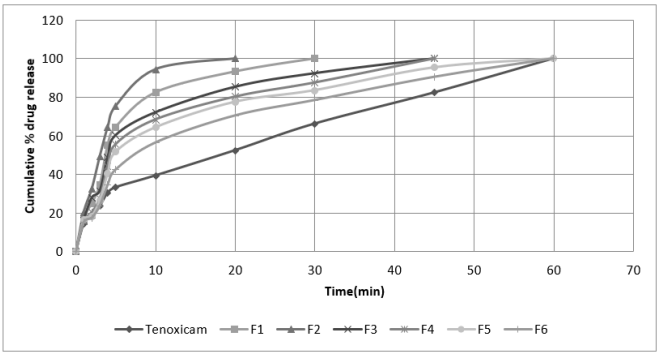

Figure 9: The cumulative% TX release of the pure drug and all the prepared formulae in phosphate buffer pH6.8 at 37ºC.

within 20 minutes, showed the most significant dissolution rate compared to pure TX study.

CONCLUSION

From this study, we can conclude that both the technique used and the ratios of drug to co-former are essential in co-crystal preparation. The formula with the ratio (1:2) TX: paracetamol prepared by solvent evaporation technique showed the best results in enhancing drug solubility and dissolution. The formation of TX-paracetamol co-crystals due to hydrogen bonding was confirmed by XRD and FTIR studies. In conclusion, solvent evaporation technique and the ratio of (1:2) TX:paracetamol are more efficient in enhancing drug solubility than grinding method and other ratios used, respectively.

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REFERENCES

1. Sareen S, Joseph L, Mathew G. Improvement in solubility of poor water-soluble drugs by solid dispersion. Int J Pharm Investig. 2012;2(1):7-12.
2. Savjani KT, Gajjar AK, Savjani JK. Drug solubility: importance and enhancement techniques. Int Sch Res Netw. 2012;2012:1–10.
3. Sathisaran I, Dalvi SV. Engineering co-crystals of poorly water-soluble drugs to enhance dissolution in aqueous medium. Pharmaceutics. 2018;10(3):1–74.
4. Gajda M, Nartowski KP, Pluta J, Karolewicz B. Continuous, one-step synthesis of pharmaceutical co-crystals via hot melt extrusion from neat to matrix-assisted processing – State of the art. Int J Pharm. 2019;558:426–440.
5. Imtihani HN, Agnes Nuniek W, Setyawan D, Hendradi E. Improvement of dissolution properties through acyclovir - Succinic acid co-crystal using solvent evaporation technique. Int J Drug Deliv Technol. 2017;7(4):304–309.
6. Ramkanth S, Jayaprakash S, Vimalakannan T. Formulation and evaluation of monolithic drug in adhesive type patch containing Tenoxicam. Int J Pharma Sci Res. 2015;6(04):654–659.
7. Patel JR, Carlton RA, Yuniatine FNU, Needham TE, Wu L. Preparation and structural characterization of amorphous spray-dried dispersions of tenoxicam with enhanced dissolution. J Pharm Sci. 2012;101(2):641–663.
8. Ibrahim MA. Tenoxicam-kollicoat ir binary systems: physicochemical and biological evaluation. Acta Pol Pharm - Drug Res. 2014;71(4):647–659.
9. Bolla G, Sanphui P, Nangia A. Solubility advantage of tenoxicam phenolic co-crystals compared to salts. Cryst Growth Des. 2013;13(5):1-16.
10. Mounika P, Raj SV, Divya G, Gowramma A, Vijayamma G, Rangampet A, et al. Preparation and characterization of novel co-crystal forms of fexofenadine. Int J Innov Pharm Res. 2015;6(1):458–463.
11. Othman MF, Anuar N, Ad Rahman S, Ahmad T NA. Cocrystal screening of ibuprofen with oxalic acid and citric acid via grinding method. In: IOP Conference series: Materials science and engineering. 2018. p. 358
12. Budiman A, Megantara S, Apriliani A. Solid dosage form development of glibenclamide-aspartame co-crystal using the solvent evaporation method to increase the solubility of glibenclamide. Int J Appl Pharm. 2019;11(3):150–154.
13. Shinde U, Desai H, Martis EAF, Amin P. Co-crystals of gliclazide : formulation and characterisation. Am J PharmTech Res. 2017;7(2):217–235.
14. Flanner H, Moore JW. Mathematical comparison of curves with an emphasis on dissolution profiles. Pharm Technol. 1996;20:64–74.
15. Negi LM, Chauhan M, Garg AK. Nano-appended transdermal gel of tenoxicam via ultradeformable drug carrier system. J Exp Nanosci. 2013;8(5):657–669.
16. Faroongsarng D, Kadejinda W, Sunthornpit A. Thermal behavior of a pharmaceutical solid acetaminophen doped with p-aminophenol. AAPS PharmSciTech. 2000;1(3):1–7.