Comparative in vitro dissolution studies of fixed-dose combination formulations: analgesic tablets of acetaminophen/caffeine

Medina-López J R*, Sánchez-Badajos S, Carreto-Jiménez R M, García-Hernández P, Contreras-Jiménez J M

Departamento Sistemas Biológicos, Universidad Autónoma Metropolitana-Xochimilco, Mexico City, Mexico

Article History: Received on: 15 Jan 2021 Revised on: 20 Feb 2021 Accepted on: 23 Feb 2021

Keywords: Acetaminophen, Caffeine, Flow-through cell apparatus, Generic formulations, USP Apparatus 4

ABSTRACT

A simple and rapid UV derivative method with zero-crossing determinations was developed for estimation of acetaminophen (ACE) and caffeine (CAF) in fixed-dose combination formulations. The first-derivative of standard solutions of both drugs were used and ACE and CAF were quantified at 273.0 and 216.5 nm, respectively. The method was validated, and it was applied to dissolution studies with the USP Apparatus 2 and flow-through cell (USP Apparatus 4). Dissolution profiles comparisons (generic vs reference) were carried out with model-independent and model-dependent approaches. Mean dissolution time and dissolution efficiency were calculated and significant differences, in almost all calculated parameters, were found (p<0.05). Weibull, logistic, Gompertz, and Probit models were used to fit dissolution data and Probit was the best-fit model that describes the in vitro dissolution performance of ACE and CAF. Using t_{50} data, derived from this fit, dissolution profiles of ACE in USP Apparatus 2 were significant different (p<0.05). The proposed UV derivative method generates reliable information that can be compared with published results. Dissolution studies of fixed-dose combination formulations are important because quality of generic drug products depends on quality of references. It is essential to maintain a post-marketing evaluation of formulations with analgesic drugs mixed with CAF to offer the population high quality medicines.

*Corresponding Author
Name: Medina-López J R
Phone: +52-5554837000
Email: rmlopez@correo.xoc.uam.mx

ISSN: 0975-7538
DOI: https://doi.org/10.26452/ijrps.v12i2.4634

INTRODUCTION

Acetaminophen (ACE) has antipyretic, analgesic, and weak anti-inflammatory actions, it is sparingly soluble in water and prone to dissolution and bioavailability problems (Hamed et al., 2017). Due to its high solubility and low permeability ACE is considered as a class III drug (Kalanizzi et al., 2006). Caffeine (CAF) is a stimulant of the central nervous system (Sawynok and Yaksh, 1993) and it has been added to non-opioid drugs to improve the analgesic effects (López et al., 2006). Chemical structures of ACE and CAF are shown in Figure 1.

Dissolution studies are usually carried out with USP basket and paddle apparatuses (USP Apparatus 1 and 2, respectively). Dissolution test of fixed-dose combination formulations of ACE/CAF is described in the USP (United States Pharmacopeial Convention, 2019). The USP Apparatus 2 at 100 rpm with 900 ml of water and chromatographic (HPLC) determination should be used. Several authors have pointed out that slow agitation
rates are preferable to develop a discriminative dissolution method (Shah et al., 1992). By the previously reported information there is a monograph that suggests the waiver of bioequivalence studies of ACE tablets by in vitro dissolution studies (Kalantzi et al., 2006) however, for fixed drug combination products where class I or III drugs are combined with any other class of drug a biowaiver approach is not applicable (FDA, 2017).

![Chemical structure of acetaminophen (left) and caffeine (right)](image)

The flow-through cell method (USP Apparatus 4) has many advantages to study the in vitro dissolution performance of active pharmaceutical ingredients (API) under a particular hydrodynamic environment (Looney, 1997; Brown, 2005). Several authors have reported in vitro/in vivo correlation with dissolution methods where the USP Apparatus 4 is used (ichi Jinno et al., 2008; Jantratid et al., 2009; Fang et al., 2010). Despite the advantage of flow-through cell to study the dissolution performance of drugs, only information about in vitro release of ACE/CAF from nanofibers and a modified dissolution procedure (Illangakoon et al., 2014) as well as with pharmaceutical formulations and the USP Apparatus 1 (Williams et al., 2010) has been reported.

Analytical methods to quantify drug mixtures in dissolution studies are mainly based on HPLC determinations however, HPLC methods are not friendly to the environment due to the high generation of toxic waste in addition to the use and maintenance of expensive equipment. UV derivative methods are an efficient alternative to identify and quantify mixtures of two or more drugs in a similar way as HPLC methods. Some UV derivative methods have been developed to dissolution studies of fixed-dose combination formulations (Medina et al., 2013, 2016; Medina-López et al., 2020).

Generic drugs are off-patent formulations that contain the same API, and the same dose, as reference drug product (Ruiz et al., 2012). These formulations must demonstrate the same quality and efficacy as the reference products. A variety of comparative in vitro dissolution studies with generic tablets of only one API have recently been published (Varillas et al., 2020; Usman et al., 2020) whereas that some authors have reported the comparison of generic drugs and reference with not entirely favorable results (Akdag et al., 2020; Alvarado et al., 2020). Dissolution studies with mixtures of non-steroidal anti-inflammatory drugs (NSAIDs) and CAF are scarce.

The aim of this study was to develop a UV derivative method with zero-crossing determinations to quantify ACE and CAF in fixed-dose combination formulations and to use the analytical procedure in a comparative in vitro dissolution study of analgesic tablets. The USP Apparatuses 2 and 4 with 0.1 M phosphate buffer pH 7.4, as dissolution medium, were used. Dissolution profiles of reference and a generic formulation were compared by model-independent and model-dependent approaches. By knowing the release performance of reference, it is possible to design better generic formulations.

**MATERIALS AND METHODS**

**Chemicals**

ACE and CAF standards were purchased from Sigma-Aldrich Co. (St. Louis MO, USA). Sodium phosphate monobasic and dibasic crystals were purchased from J.T.Baker-Mexico (Xalostoc-Mexico). Two ACE/CAF fixed-dose combination formulations (500/50 mg tablets) were used in this study. One of them was the Saridon® product (Bayer de Mexico S.A. de C.V., Mexico). Mexican health regulatory agency (Cofepris) has established this commercial brand as the reference to be used in bioequivalence studies (Cofepris, 2020). The second drug product was a generic formulation. The R and G letters were assigned to them, respectively.

**Spectrophotometric conditions**

ACE and CAF were simultaneously determined by a proposed UV derivative method. A double beam UV/Vis spectrophotometer (Perkin Elmer Lambda 35, Waltham MA, USA) with 1 cm quartz cells was utilized. The operating conditions for UV analysis were first-derivative mode (1D) with scan speed 240 nm/min, slit width 2 nm and sampling interval 1 nm.

**Standard calibration curves**

Stock solutions of ACE and CAF in 0.1 M phosphate buffer pH 7.4 (1 mg/ml) were separately prepared. Five standard solutions of each drug in 0.1 M phosphate buffer pH 7.4 were prepared. The concentration range of ACE was 10–30 μg/ml and for CAF was 0.5–5 μg/ml.

Then, the zero order spectra taken from 200 to 350 nm, using 1 cm quartz cells, were recorded, and stored. With these spectra the 1D of each solution...
was calculated.

**Linearity, precision, and accuracy**

Linearity was tested with the preparation of two standard calibration curves of each drug. All solutions were analyzed according to the procedures described above and mean data were used for mathematical treatments. Linear regression analysis was calculated and an analysis of variance (ANOVA) of each regression was carried out. The 95% confidence intervals (CI95%) for intercepts were calculated. Precision was verified with the calculation of coefficient of variation (CV) at each concentration level. Accuracy was confirmed with the analysis of three synthetic mixtures at following concentrations: 12, 18, and 27 μg/ml of ACE and 1.5, 3, and 4 μg/ml of CAF. Mixtures were analyzed according to the proposed UV derivative method. Found vs. added concentrations were plotted and linear regression analysis were calculated. Then, CI95% for slopes and intercepts were determined.

**Uniformity of dosage units and assay tests**

Both tests were performed to R and G formulations according the USP procedures (United States Pharmacopeial Convention, 2019).

**Dissolution profiles**

**USP Apparatus 2**

Dissolution profiles of ACE and CAF were obtained in a USP Apparatus 2 (Sotax AT-7 Smart, Switzerland) at 75 rpm using 900 ml of 0.1 M phosphate buffer pH 7.4 at 37.0±0.5 °C as dissolution medium. Prior to use, the dissolution medium was deaerated by vacuum and transferred into the dissolution vessels. At 10, 20, 30, 45, and 60 min a volume of 3 ml was withdrawn, and it was filtered with nitrocellulose filters (Millipore). The amounts of ACE and CAF dissolved were determined with standard calibration curves of each drug.

**USP Apparatus 4**

Dissolution profiles of ACE and CAF were obtained in a USP Apparatus 4 (Sotax CE6, Sotax AG, Switzerland) with 22.6 mm cells (i.d.). Laminar flow (generated with a bed of 6 g of glass beads) was used.

The degassed dissolution medium, 0.1 M phosphate buffer pH 7.4 at 37.0±0.5 °C was pumped at a flow rate of 16 ml/min. An open system was used, without recycling the dissolution medium. At 10, 20, 30, 45, and 60 min a volume of 3 ml was withdrawn, and it was filtered with nitrocellulose filters. For every trial, standard calibration curves were prepared.

**Data analysis**

Dissolution profiles of ACE and CAF from R and G formulations were compared by model-independent and model-dependent approaches. For model-independent comparisons $f_2$ similarity factor, mean dissolution time (MDT), and dissolution efficiency (DE) were calculated. Similarity factor was calculated according to equation previously published (Moore and Flanner, 1996). Similar dissolution profiles were considered if $f_2 = 50$ to 100. MDT and DE were compared by a Student’s $t$-test and significant differences were considered if $p<0.05$. For model-dependent comparisons, dissolution data were fitted to Hyperbola equation and with $a$ and $b$ parameters $t$50% values were calculated. Additionally, dissolution data of ACE and CAF were fitted to Weibull, logistic, Gompertz, and Probit models (Zhang et al., 2010). Mathematical equation of each model is shown in Table 1. The model with the highest adjusted determination coefficient ($R^2_{adj}$) and lowest Akaike Information Criterion (AIC) was chosen as the best-fit model (Yuksel et al., 2000). Data analysis was carried out using the Excel add-in DDSolver program (Zhang et al., 2010).

**RESULTS AND DISCUSSION**

**UV derivative method**

The adequate identification and quantification of ACE and CAF with the proposed UV derivative method is shown in Figure 2.

As shown in Figure 2A, ACE and CAF cannot be quantified with direct absorbance data because the zero-order spectrum of the mix solution shows an overlapped spectrum. The zero-crossing points to quantify ACE and CAF were identified at 273.0 and 216.5 nm, respectively. At these wavelengths, all analytical
Table 2: Results of quality control pharmacopeial tests

| USP test          | Reference | Generic |
|-------------------|-----------|---------|
|                   | ACE       | CAF     | ACE     | CAF     |
| Uniformity of dosage units (min-max%)† | 90.58-106.27 | 90.65-102.86 | 96.79-105.49 | 96.35-103.86 |
| Assay (%)‡        | 101.48    | 102.66  | 101.95  | 105.20  |

†n=10. ‡n=3

Table 3: Model-independent parameters of ACE and CAF

| USP | Drug | Code | Q₆₀ (%) | MDT (min) | DE (%) | f₂ |
|-----|------|------|---------|-----------|--------|----|
| 2   | ACE  | R    | 99.63±1.49 | 6.71±0.34 | 88.50±1.43 | 61.71 |
|     |      | G    | 97.13±0.79 | 7.65±0.37 | 84.70±0.46* |           |
|     | CAF  | R    | 106.66±1.42 | 6.50±0.35 | 95.06±1.08 | 41.69 |
|     |      | G    | 91.67±0.84* | 7.03±0.38 | 80.90±0.60* |           |
| 4   | ACE  | R    | 102.31±2.75 | 10.33±0.70 | 84.47±1.80 | 57.80 |
|     |      | G    | 109.03±1.77 | 9.66±0.68 | 91.32±1.04* |           |
|     | CAF  | R    | 74.20±2.03  | 7.61±0.92 | 64.51±1.06 | 30.50 |
|     |      | G    | 109.63±1.46* | 11.81±0.33* | 88.00±0.89* |           |

Mean value±standard error mean, n=12, *p<0.05. Q₆₀: drug dissolved at 60 min. MDT: mean dissolution time. DE: dissolution efficiency.

Table 4: Hiperbola parameters and t₅₀% data derived from this fit

| USP | Drug | Code | a    | b    | R²   | t₅₀% (min) |
|-----|------|------|------|------|------|------------|
| 2   | ACE  | R    | 101.57 | 1.22 | 0.9990 | 1.15±0.18 |
|     |      | G    | 104.79 | 3.36 | 0.9961 | 2.98±0.33* |
|     | CAF  | R    | 107.91 | 0.96 | 0.9987 | 0.80±0.17 |
|     |      | G    | 97.20  | 2.53 | 0.9966 | 2.59±0.37* |
| 4   | ACE  | R    | 114.98 | 6.29 | 0.9923 | 4.75±0.36 |
|     |      | G    | 122.13 | 5.77 | 0.9944 | 3.89±0.36 |
|     | CAF  | R    | 78.37  | 2.75 | 0.9891 | 5.04±0.39 |
|     |      | G    | 122.60 | 7.07 | 0.9975 | 4.77±0.34 |

Mean±SEM, n=12, *p<0.05

signals were proportional to drugs concentrations.

**Linearity, precision, and accuracy**

Linear regression equations to test linearity and accuracy of ACE and CAF are shown in Figure 3. To test linearity all linear regressions were significant (p<0.05). CI₉₅% for intercepts were: -0.019 to 0.0018 for ACE and -0.0046 to 0.023 for CAF. Considering both drugs the CV ranged from 0.39 to 2.53%. To test accuracy CI₉₅% for intercepts and slopes were: -3.82 to 3.70 and 0.82 to 1.20 for ACE and -0.52 to 0.38 and 0.87 to 1.17 for CAF. Considering these results, the proposed UV derivative method was a good alternative to determine the in vitro dissolution performance of ACE and CAF in fixed-dose combination formulations.

**Uniformity of dosage units and assay**

Both commercial formulations met the uniformity of dosage units and assay tests. The percent of ACE and CAF content ranged from 85 to 115% and the assay test was between 90 and 110%. Results are shown in Table 2.

**Dissolution profiles**

Dissolution profiles of ACE and CAF obtained with USP Apparatuses 2 and 4 are shown in Figure 4. To compare dissolution profiles (reference vs. generic formulation) model-independent parameters were calculated. Percent of drug dissolved at 60 min (Q₆₀), MDT, DE, and f₂ similarity factor are shown in Table 3. Considering f₂ factor, only dissolution profiles of ACE were similar (f₂>50). Some other comparisons match this result. All comparisons of CAF, in USP Apparatus 4, shown
Table 5: Criteria used to choose the best-fit model

| USP | Drug | Code | Weibull | Logistic | Gompertz | Probit |
|-----|------|------|---------|----------|----------|--------|
| 2   | ACE  | R    | 0.9989  | 0.9992   | 0.9992   | 0.9992 |
|     |      | G    | 0.9984  | 0.9987   | 0.9987   | 0.9988 |
|     | CAF  | R    | 0.9983  | 0.9990   | 0.9990   | 0.9990 |
|     |      | G    | 0.9971  | 0.9979   | 0.9979   | 0.9979 |
| 4   | ACE  | R    | 0.9903  | 0.9930   | 0.9929   | 0.9931 |
|     |      | G    | 0.9957  | 0.9967   | 0.9967   | 0.9967 |
|     | CAF  | R    | 0.9811  | 0.9874   | 0.9874   | 0.9875 |
|     |      | G    | 0.9967  | 0.9976   | 0.9975   | 0.9977 |

Mean value, n=12

Table 6: Probit parameters and t_{50\%} data derived from this fit

| USP | Drug | Code | α     | β     | F_{max} | t_{50\%} (min) |
|-----|------|------|-------|-------|---------|----------------|
| 2   | ACE  | R    | -0.44 | 1.22  | 215.41  | 1.30           |
|     |      | G    | -1.73 | 2.44  | 104.12  | 4.53*          |
| 4   | CAF  | R    | -2.31 | 2.84  | 385.71  | 5.88           |
|     |      | G    | -1.43 | 1.55  | 162.07  | 4.84           |

Mean values, n=12, *p<0.05

significant differences (p<0.05). On the other hand, the in vitro dissolution performance of ACE and CAF was slower with the flow-through cell apparatus than the USP Apparatus 2. This kind of behavior has been reported by some authors due to hydrodynamic environment that USP Apparatus 4 generates over the dosage form (Looney, 1997; Brown, 2005; Medina et al., 2014).

To compare dissolution profiles with a model-dependent approach and after the adjustment of dissolution data to Hyperbola model the t_{50\%} values of ACE and CAF were calculated. Results are shown in Table 4. With these data, similar dissolution profiles of both drugs were found only with the USP Apparatus 4 (p>0.05).

For a complete model-dependent comparison scheme dissolution data of ACE and CAF were adjusted to common equations. Weibull, logistic, Gompertz, and Probit equations were used. Results are shown in Table 5.

As can be seen in Table 5 and considering the criteria to choose the best-fit model, only dissolution performance of ACE in USP Apparatus 2 and CAF in USP Apparatus 4 were well described by the same equation (Probit model). To compare dissolution profiles of ACE and CAF, under previously described conditions, the t_{50\%} values derived from Probit model were calculated. Results are shown in Table 6. Significant differences were found only with ACE data (p<0.05).

Results do not match between comparison approaches used. Due to diversity of the results obtained, it can be concluded that the dissolution profiles of ACE and CAF between reference and generic formulation are not similar and the drugs do not show equivalence in their in vitro release performance.

Some authors have studied the UV identification of ACE/CAF in granules (Szumilo et al., 2019), and in commercial tablets (Dinç, 1999; Vichare et al.,...
Figure 2: A) Zero-order spectra a solution of ACE (20 µg/ml), CAF (2 µg/ml) and a mixture of both drugs (MIX) at same concentrations. B) 1D of same solutions. C) 1D of standard calibration curves. Vertical lines shown the zero-crossing points at 216.5 and 273 nm.

These methods used simultaneous equations to quantify both drugs without mutual interference. In granules, more than 80% of ACE and CAF were released in less than 10 min (Szumilo et al., 2019) and both analytical methods reported for commercial tablets can be used for quality control purposes. Especially in dissolution studies, controlled release of CAF tablets has been reported by some authors (Franek et al., 2014; Tan et al., 2019) and more than 80% of drug dissolved was found between 20-24 h. On the other hand, more than 80% of CAF at 20 min was found in immediate-release formulations with a binary mixture of drugs and HPLC determination (Williams et al., 2010) as well as with a ternary mixture (Liu et al., 1999). Both dissolution methods used the USP Apparatus 1 at 100 rpm. In this work, an easier and faster procedure with derivative spectrophotometry and
zero-crossing determinations is proposed and similar results were obtained.

Linearity, precision, and accuracy were evaluated with good results. Considering these data, it is possible state that UV derivative method allows the study of the in vitro dissolution performance of ACE and CAF in fixed-dose combination formulations and significant differences between dissolution profiles of generic and reference were found. There are many potential factors that can explain the difference between the branded and their generic counterparts. Those include the manufacturer, apparatus type, surface area of a drug, surfactants, storage, dosage form and the level and type of excipients (Ameri et al., 2012).

Figure 3: A and B) Standard calibration curves (n=2). C and D) Synthetic mixtures (n=3)

Official dissolution test for ACE/CAF tablets describes the use of USP Apparatus 2 at 100 rpm and 900 ml of water as dissolution medium (Q>75% at 60 min) (United States Pharmacopeial Convention, 2019). In this comparative dissolution study, we use the USP Apparatus 2 at 75 rpm and the flow-through cell apparatus with a controlled pH dissolution medium (0.1 M phosphate buffer pH 7.4) with the aim of evaluate the commercial formulations under a hydrodynamic environment more similar to the gastrointestinal tract. Using our conditions, ACE and CAF reached more than 75% of drug dissolved at 60 min excepting CAF in USP Apparatus 4 (74.20%). Several authors have reported that the proper medium and rotational speed of the paddle is important in assuring that the dissolution test is useful and discriminatory (Shah et al., 1992). Moreover, the maximum fluid velocities in the flow-through cell apparatus at standard operating conditions are expected to be considerably lower than those found in the paddle or basket apparatuses (D’Arcy et al., 2010).

Although hydrodynamic environments of USP Apparatus 2 and 4 are different, dissolution profiles comparison helps us to identify if there is an equivalence between dissolution USP Apparatuses. Under certain dissolution conditions, propranolol-HCl (a high solubility drug) has been shown equivalence in its in vitro release performance between USP
basket apparatus and flow-through cell (Medina-Lopez et al., 2019). On the other hand, the model-independent parameters MDT and DE have been suggested as suitable parameters to compare dissolution profiles (Podczeck, 1993; Anderson et al., 1998) and to establish an in vitro/in vivo correlation (Cardot et al., 2007).

Several methods are available to elucidate dissolution data as a function of time, but its dependence on dosage form characteristics can best be deduced by using generic equations which mathematically translates the dissolution curves in the function of other parameters related to delivery device (Lokhandwala et al., 2013). Mathematical models describe the dissolution curves as a function of only a few parameters that can be statistically compared (Adams et al., 2002).

While USP Apparatuses 1 and 2 are currently the most popular methods to study the in vitro dissolution performance of drugs, both methods are operated under closed finite sink conditions and cannot mimic the environment of the gastrointestinal tract (Gao, 2009). The flow-through cell apparatus has the advantage of generating a hydrodynamic environment similar to that found inside the digestive system (Looney, 1997). It is important to perform a post-marketing monitoring to ensure safety and efficacy of ACE/CAF fixed-dose combination formulations with all available analytical methodologies. ACE is considered a hepatotoxic drug and it is easily accessible in several formulations as an over-the-counter medication (Yoon et al., 2016). There are many drug products combined with CAF to enhance the analgesic properties of AINEs and dissolution profiles comparison of AINEs/CAF generic formulations are scarce.

**CONCLUSION**

The proposed UV derivative method was applied for determination of ACE and CAF in fixed-dose combination formulations. The method was useful for determination of in vitro dissolution performance of
both drugs in reference and generic formulations. It is important to carry out bioequivalence studies with ACE/CAF tablets to relate dissolution data with its in vivo behavior. It is essential to maintain a post-marketing evaluation of formulations with analgesic drugs mixed with CAF to offer the population high quality medicines. More research is needed in this field.

**Funding Support**

The authors declare that they have no funding support for this study.

**Conflict of Interest**

The authors declare that they have no conflict of interest for this study.

**REFERENCES**

Adams, E., Coomans, D., Smeyers-Verbeke, J., Massart, D. L. 2002. Non-linear mixed effects models for the evaluation of dissolution profiles. *International Journal of Pharmaceutics*, 240(1-2):37–53.

Akdag, Y., Gulsun, T., Izat, N., Oner, L., Sahin, S. 2020. Comparison of Dissolution Profiles and Apparent Permeabilities of Commercially Available Metformin Hydrochloride Tablets in Turkey. *Dissolution Technologies*, 27(1):22–29.

Alvarado, A. T., Muñoz, A. M., Miyasato, J. M., Alvarado, E. A., Loja, B., Villanueva, L., Pineda, M., Bendezú, M., Palomino-Jhong, J. J., Garcia, J. A. 2020. In Vitro Therapeutic Equivalence of Two Multisource (Generic) Formulations of Sodium Phenytoin (100 mg) Available in Peru. *Dissolution Technologies*, 27(4):33–40.

Ameri, M. N. A., Nayuni, N., Kumar, K. A., Perrett, D., Tucker, A., Johnston, A. 2012. The differences between the branded and generic medicines using solid dosage forms: In-vitro dissolution testing. *Results in Pharma Sciences*, 2:1–8.

Anderson, N. H., Bauer, M., Boussac, N., Khan-Malek, R., Munden, P., Sardaro, M. 1998. An evaluation of fit factors and dissolution efficiency for the comparison of in vitro dissolution profiles. *Journal of Pharmaceutical and Biomedical Analysis*, 17(4-5):811–822.

Brown, W. 2005. Apparatus 4 Flow Through Cell: Some Thoughts on Operational Characteristics. *Dissolution Technologies*, 12(2):28–30.

Cardot, J.-M., Beyssac, E., Alric, M. 2007. In Vitro–In Vivo Correlation: Importance of Dissolution in IVIVC. *Dissolution Technologies*, 14(1):15–19.

Cofepris, L. 2020. Listado actualizado de medicamentos de referencia 2020/01. Mexico, Accessed on: 21 Jan 2021.

D’Arcy, D. M., Liu, B., Bradley, G., Healy, A. M., Corrigan, O. I. 2010. Hydrodynamic and Species Transfer Simulations in the USP 4 Dissolution Apparatus: Considerations for Dissolution in a Low Velocity Pulsing Flow. *Pharmaceutical Research*, 27(2):246–258.

Dinç, E. 1999. A comparative study of the ratio spectra derivative spectrophotometry, Vierordt’s method and high-performance liquid chromatography applied to the simultaneous analysis of caffeine and paracetamol in tablets. *Journal of Pharmaceutical and Biomedical Analysis*, 21(4):723–730.

FDA 2017. Guidance for Industry. Waiver on in vivo bioavailability and bioequivalence studies for immediate-release solid oral dosage forms based on a biopharmaceutics classification system. U.S. Department of Health and Human Services, Center for Drug Evaluation and Research (CDER). Accessed on: 21 Jan 2021.

Franek, F., Holm, P., Larsen, F., Steffansen, B. 2014. Interaction between fed gastric media (Ensure Plus®) and different hypromellose based caffeine controlled release tablets: Comparison and mechanistic study of caffeine release in fed and fasted media versus water using the USP dissolution apparatus 3. *International Journal of Pharmaceutics*, 461(1-2):419–426.

Gao, Z. 2009. In Vitro Dissolution Testing with Flow-Through Method: A Technical Note. *AAPS PharmSciTech*, 10(4):1401–1405.

Hamed, S., Ayob, F. A., Alfatama, M., Doolaeanea, A. A. 2017. Enhancement of the Immediate Release of Paracetamol from Alginate Beads. *International Journal of Applied Pharmaceutics*, 9(2):47–51.

Ichin Jino, J., Kamada, N., Miyake, M., Yamada, K., Mukai, T., Odomi, M., Toguchi, H., Liversidge, G. G., Hijiki, K., Kimura, T. 2008. In vitro—in vivo correlation for wet-milled tablet of poorly watersoluble cilostazol. *Journal of Controlled Release*, 130(1):29–37.

Illangakoon, U. E., Gill, H., Shearman, G. C., Parhizkar, M., Mahalingam, S., Chatterton, N. P., Williams, G. R. 2014. Fast dissolving paracetamol/caffeine nanofibers prepared by electrospinning. *International Journal of Pharmaceutics*, 477(1-2):369–379.
Jantratid, E., Maio, V. D., Ronda, E., Mattavelli, V., Vertzoni, M., Dressman, J. B. 2009. Application of biorelevant dissolution tests to the prediction of in vivo performance of diclofenac sodium from an oral modified-release pellet dosage form. European Journal of Pharmaceutical Sciences, 37(3-4):434–441.

Kalantzi, L., Reppas, C., Dressman, J. B., Amidon, G. L., Junginger, H. E., Midha, K. K., Shah, V. P., Stavchansky, S. A., Barends, D. M. 2006. Biowaiver monographs for immediate release solid oral dosage forms: Acetaminophen (paracetamol). Journal of Pharmaceutical Sciences, 95(1):4–14.

Liu, X. Z., Liu, S. S., Wu, J. F., Fang, Z. L. 1999. Simultaneous monitoring of aspirin, phenacetin and caffeine in compound aspirin tablets using a sequential injection drug-dissolution testing system with partial least squares calibration. Analytica Chimica Acta, 392(2-3):273–281.

Lokhandwala, H., Deshpande, A., Deshpande, S. 2013. Kinetic modeling and dissolution profiles comparison: an overview. International Journal of Pharma and Bio Sciences, 4(1):728–737.

Looney, T. J. 1997. USP Apparatus 4 - Applying the Technology. Dissolution Technologies, 4(2):16–18.

López, J. R. M., Domínguez-Ramírez, A. M., Cook, H. J., Bravo, G., Díaz-Reval, M. I., Déciga-Campos, M., López-Muñoz, F. J. 2006. Enhancement of antinociception by co-administration of ibuprofen and caffeine in arthritic rats. European Journal of Pharmacology, 544(1-3):31–38.

Medina, J. R., García, C. A., Hurtado, M., Domínguez-Ramírez, A. M. 2016. Simultaneous determination of ketoprofen and acetaminophen in fixed-dose combination formulations by first-order derivative spectroscopy: application to dissolution studies. International Journal of Pharmacy and Pharmaceutical Sciences, 8(2):244–248.

Medina, J. R., Miranda, M., Hurtado, M., Domínguez-Ramírez, A. M., Ruiz-Segura, J. C. 2013. Simultaneous determination of trimethoprim and sulfamethoxazole in immediate-release oral dosage forms by first-order derivative spectroscopy: Application to dissolution studies. International Journal of Pharmacy and Pharmaceutical Sciences, 5(Supp4):505–510.

Medina, J. R., Salazar, D. K., Hurtado, M., Cortés, A. R., Domínguez-Ramírez, A. M. 2014. Comparative in vitro dissolution study of carbamazepine immediate-release products using the USP paddles method and the flow-through cell system. Saudi Pharmaceutical Journal, 22(2):141–147.

Medina-López, J. R., Pineda, J. G. P., et al. 2020. Derivatives of the ratio spectra for determination of acetylsalicylic acid, acetaminophen, and caffeine in fixed-dose combination formulations: application to dissolution studies. International Journal of Applied Pharmaceutics, 12(6):253–257.

Medina-Lopez, R., Reyes-Ibarra, A. D., Hurtado, M. 2019. In Vitro Equivalence Study of Propranolol-HCl tablets Using USP Basket Apparatus and Flow-Through Cell Method. Latin American Journal Of Pharmacy, 38(1):92–101.

Moore, J. W., Flanner, H. H. 1996. Mathematical comparison of dissolution profiles. Pharmaceutical technology, 20(6):64–74.

Podczeck, F. 1993. Comparison of in vitro dissolution profiles by calculating mean dissolution time (MDT) or mean residence time (MRT). International Journal of Pharmaceutics, 97(1-3):93–100.

Ruiz, M. E., Gregorini, A., Talevi, A., Volonté, M. G. 2012. Dissolution Studies of Generic Medications: New Evidence of Deviations from the Transitivity Principle. Dissolution Technologies, 19(1):13–24.

Sawynok, J., Yaksh, T. L. 1993. Caffeine as an analgesic adjuvant: a review of pharmacology and mechanisms of action. Pharmacological Reviews, 45(1):43–85.

Shah, V. P., Gurbarg, M., Noory, A., Dighe, S., Skelly, J. P. 1992. Influence of Higher Rates of Agitation on Release Patterns of Immediate-Release Drug Products. Journal of Pharmaceutical Sciences, 81(6):500–503.

Szumilo, M., Belniak, P., Kasperek-Nowakiewicz, R., Holody, E., Poleszak, E. 2019. Comparative dissolution studies on granules with acetaminophen and caffeine using the basket and paddle methods with simultaneous spectrophotometric determination of active substances. Current Issues in Pharmacy and Medical Sciences, 32(4):219–224.

Tan, S., Ebrahim, A., Langrish, T. 2019. Controlled release of caffeine from tablets of spray-dried casein gels. Food Hydrocolloids, 88:13–20.

United States Pharmacopeial Convention 2019. The United States Pharmacopoeia: USP 42: The National Formulary: NF 37. Rockville, MD. page 52.

Usman, S., Saeed, A., Fatima, S., Ramesh, V., Shah, F., Islam, Q. 2020. In Vitro Bioequivalence of Pregabal Capsules (150 mg): An Alternative to In Vivo Bioequivalence Studies. Dissolution Technologies, 27(4):24–31.

Varillas, M. A., Brevedan, M. I., Vidal, N. L. G. 2020. Critical Quality Attributes and Pharmaceutical Equivalence Assessment of Allopurinol Tablets in Argentina. Dissolution Technologies, 27(4):15–
22.

Vichare, V., Mujgond, P., Tambe, V., Dhole, S. N. 2010. Simultaneous spectrophotometric determination of paracetamol and caffeine in tablet formulation. *International Journal of PharmTech Research, 2*(4):2512–2516.

Williams, H. D., Barrett, D. A., Ward, R., Hardy, I. J., Melia, C. D. 2010. A liquid chromatography method for quantifying caffeine dissolution from pharmaceutical formulations into colloidal, fat-rich media. *Journal of Chromatography B, 878*(21):1739–1745.

Yoon, E., Babar, A., Choudhary, M., Kutner, M., Pyrsopoulos, N. 2016. Acetaminophen-Induced Hepatotoxicity: a Comprehensive Update. *Journal of Clinical and Translational Hepatology, 4*(2):131–142.

Yuksel, N., et al. 2000. Comparison of in vitro dissolution profiles by ANOVA-based, model-dependent and -independent methods. *International Journal of Pharmaceutics, 209*(1-2):57–67.

Zhang, Y., Huo, M., Zhou, J., Zou, A., Li, W., Yao, C., Xie, S. 2010. DDSolver: An Add-In Program for Modeling and Comparison of Drug Dissolution Profiles. *The AAPS Journal, 12*(3):263–271.