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Spectroscopic *In-Vitro* Drug-Drug Interaction Studies of Amoxicillin and Paracetamol Solid Dosage Forms

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Abstract **Original Research Article**

Potential drug-drug interactions (DDIs) are a public health concern in clinical therapy and drug development because of their contribution to actual adverse drug reactions (ADRs) or events. The study aimed to quantify the in-vitro drug-drug interaction of amoxicillin (AMX) and paracetamol (PCM) formulations which are commonly co-administered. Determinations were carried out by spectroscopic methods – zero-order and derivative spectroscopy. The data were analyzed using Microsoft Excel, 2016. The UV-Spectra of AMX and PCM showed two distinctive peaks at 240 nm and 260 nm were observed for AMX, while for PCM at 290nm and 340 nm, with maxima at 240 nm and 340 nm for AMX and PCM respectively. Absorption measurements were taken at the maximum wavelength of 290 nm and 240 nm for PCM and AMX respectively. The content of both drugs and potential DDIs of AMX and PCM were assayed by zero- and 3rd-derivative spectroscopy. The calibration curves for AMX and PCM standards showed good linearity at concentrations, with corresponding correlation coefficients (R) being 0.999 and 0.998. The intercept and slope for AMX were -0.0148 and 0.0732 respectively, while for PCM values were 0.0071 and 0.148. The content of PCM and AMX in the formulations was 98.40 ± 0.44% and 96.04 ± 0.29% respectively. The joint-drug-dissolution studies for AMX and PCM interaction (DDI) were obtained with the 3rd-order derivative spectroscopy at 210 nm (zero-crossing for PCM) and 290 nm (zero-line crossing for AMX) respectively - which showed overlapping spectra for their zero-order derivative spectra. No significant difference was observed in the in-vitro drug release profiles for AMX and PCM, while comparative profiles were observed to have a very strong correlation between individual-release and joint-release profiles.

Keywords: Spectroscopy, In-Vitro, Drug-Drug Interaction, Amoxicillin, Paracetamol.

INTRODUCTION

Guidelines for drug interaction studies are usually formulated and promulgated by the United States Food and Drug Administration and the European Medicines Agency, including suggestions for both in vitro and in vivo investigations to analyze potential drug interactions [1]. Identifying potential drug-drug interactions (DDIs) has long been a concern in clinical therapy and drug development. Deep learning techniques have recently been developed to predict DDIs but require a significant number of samples, and known DDIs are uncommon [2]. The FDA issued two recommendations in 2020: in vitro drug interaction studies guidance for the industry and clinical drug interaction studies guidance for the industry involving cytochrome P450 enzyme and transporter-mediated drug interactions, respectively, which updated the 2017 guidelines [3].

Adverse drug reactions (ADRs) are a global public health concern that should be addressed because of their impact on mortality, morbidity, and healthcare costs. Drug-drug interactions (DDIs) contribute significantly to ADRs [4]. Many of these interactions leading to major ADRs are poorly reported in developing nations [5]. Physicians and other healthcare providers, especially Pharmacists should get ongoing education and training to recognize and understand the ADRs linked with DDIs [4, 6, 7]. DDIs occur when one drug influences the pharmacokinetics of another or its metabolites when they are administered together. Pharmacokinetic DDIs are evaluated during clinical trials to examine both the safety and pharmacology of a novel drug. DDIs and associated ADRs are a common source of drug market withdrawals and, hence, are of significant concern early in drug design and development [8, 9]. Studies have shown that the probability of DDIs increases with the number of medications co-

administered, old age [10], certain food and herb consumption [11, 12], and drug metabolism associated with genetic polymorphism [13, 14], among others. This indicates the need to assess the potential DDIs of Amoxicillin-Paracetamol (AMX - PCM) which are frequently co-administered in the management of fever [15-17] and infections caused by susceptible organisms [18, 19].

Several research has quantified DDIs of commonly co-administered medications in vitro, using the RP-HPLC techniques [20], FTIR, and ^{1}HNMR spectroscopy [21]. These techniques are highly professional and expensive, hence developing a simpler in vitro spectroscopic method to determine the potential DDIs of frequently co-administered medications is vital due to the significant role they play in ADRs [22]. Chromatrophaphic [23-25], UV-spectroscopic [26-28], derivative spectroscopy [29], titrimetric [30, 31], elemental analysis [32, 33], etc, are some simple valuable techniques that can be employed in the real-time quantification of DDIs. The current study aimed to spectroscopically quantify the potential in-vitro drug-drug interaction of AMX and PCM solid dosage forms commonly co-administered among the Nigerian population.

MATERIALS AND METHODS

Reagent and Equipment

All the chemicals used were of analytical grade. Pure Amoxicillin trihydrate powder (99.68% secondary standard), and pure paracetamol powder (99.88%, secondary standard), were donated by Primex Nigeria Ltd., Ikeja, Lagos, and Sagar Vitaceuticals Nigeria Limited, Ikeja Lagos respectively. Innovator brands of Amoxicillin (Amoxil® - Beecham) and Paracetamol (Panadol® – GlaxoSmithKline) were purchased from KETO DEVINE Pharmacy, Amassoma, Bayelsa State, Nigeria. Potassium dihydrogen orthophosphate (anhydrous 98%) (Loba Chemie PVT LMT), Disodium hydrogen orthophosphate (J.T Baker USA), distilled water. Spectrumlab 752pro UV-VIS spectrophotometer, Dissolution Apparatus.

Preparation of Dissolution Medium and Determination of Maximum Wavelength

Phosphate buffer pH 6.8: The buffer was prepared by dissolving 28.80g of disodium hydrogen orthophosphate and 11.45g of potassium dihydrogen orthophosphate in sufficient water to produce 1000ml, following the British Pharmacopoeia guidelines [34]. To 100 mg paracetamol reference standard in a 100 mL volumetric flask 10 mL phosphate buffer solution was added to dissolve the powder and made to mark with the buffer (stock solution 1000 ug/mL). An aliquot of 1 mL of this solution was then transferred to a 20 mL volumetric flask and made to the mark with the same buffer to obtain a concentration of 50 µg mL^{-1}. The resultant solution was then scanned in the UV region, 200 – 380 nm. The wavelength with the highest absorbance was noted as the maximum wavelength (λ_{max}). Also, an equivalent weight of 50 mg amoxicillin standard powder was dissolved in a 10 mL buffer solution in a 50 mL volumetric flask. This was shaken gently for 1 minute and then made to mark with the phosphate buffer solution (Stock solution 1000 ug mL^{-1}). An aliquot of 0.1 mL was then transferred to a 10 ml volumetric flask and made to mark with the buffer solution to obtain a concentration of 10.0 ug mL^{-1}. This was then scanned in the UV region, 200-380 nm. The wavelength with the highest absorbance was noted as λ_{max}.

Calibration curve of paracetamol and amoxicillin

The concentrations 5, 10, 15, 20, and 25 ug mL^{-1} were prepared for the calibration of paracetamol standard, while for amoxicillin were 2, 4, 6, 8, and 10 ug mL^{-1}. Both working calibration concentrations were prepared from their stock solutions. The absorbance of these concentrations was taken at the maximum wavelength of 290 nm and 240 nm for paracetamol and amoxicillin respectively. Values obtained were used to plot the calibration curves.

Assay determination of paracetamol and amoxicillin contents in formulations

Paracetamol Tablets:

An equivalent of 50 mg of powdered paracetamol tablet was transferred into a 50 mL volumetric flask and shaken gently with 30 mL of phosphate buffer solution for 3-5 minutes. This was made to the mark with the buffer solution. Aliquots of 0.05, 0.1, and 0.2 mL, were then transferred into separate 10 ml volumetric flask and diluted to mark with phosphate buffer (pH, 6.8). The absorbance of the resultant solutions was measured at 290 nm. This procedure was repeated twice and the percentage content of the paracetamol tablet was calculated from the calibration curve.

Amoxicillin Capsules:

To 50 mg equivalent of amoxicillin capsule powder in a 50 mL volumetric flask, 25 mL phosphate buffer was added and shaken gently for 3-5 minutes. This was then made to mark with the buffer solution. Aliquots of 0.1, 0.15, and 0.2 mL, were transferred into separate 10 ml volumetric flask and made to mark with phosphate buffer (pH, 6.8). The absorbance of these solutions was obtained at 240 nm (Zero Order Spectroscopy) and 210 nm (3rd Derivative Order Spectroscopy). This procedure was repeated twice and amoxicillin content in capsules was calculated from the calibration curve.

In-vitro drug-drug interaction studies

A two-phase *in-vitro* dissolution study was adopted for the drug-drug interaction studies. Firstly, separate dissolution studies of amoxicillin and paracetamol were carried out in a phosphate buffer (pH 6.8) medium and this was followed by joint in-vitro release studies of amoxicillin and paracetamol in a

phosphate buffer (pH 6.8) solution with both drugs in the same trough and amount of unreacted drugs were determined to ascertain the extent of amoxicillin-paracetamol interaction. Before the study, the maximum absorbance for amoxicillin and paracetamol in phosphate buffer pH 6.8 was determined by scanning from 200 – 380 nm. To determine the amount of each drug released at different time amplitudes a deconvolution of the UV spectra of amoxicillin and paracetamol was applied by derivative UV-spectroscopy.

Phase I:

The dissolution test was separately carried out for paracetamol tablets and amoxicillin capsules by the BP method using USP apparatus 1 (Basket method) in 6 replicates each [34]. The dissolution medium was 900 mL phosphate buffer solution (pH 6.8) which was maintained at 37 ± 0.5 ºC. At various time amplitudes - 0, 5, 10, 15, 30, 45, and 60 minutes, 5 mL aliquots of dissolution sample were withdrawn and assayed for the amount of drug released into the medium. The withdrawn volume was immediately replaced to maintain the condition of the sink. Samples were filtered, diluted with the dissolution medium, and assayed by UV spectrophotometry. The absorbance of paracetamol and amoxicillin were measured at 290 and 240 nm respectively. The amount of analyte released per time amplitude was determined from the calibration curves for amoxicillin and paracetamol [34].

Phase II:

The BP dissolution method [34] in Phase 1 was adopted. However, for each 6 replicates paracetamol tablet and amoxicillin capsule were introduced jointly in the phosphate buffer medium (pH 6.8). At various time intervals - 0, 5, 10, 15, 30, 45, and 60 minutes, 5 mL aliquots of dissolution samples were withdrawn and simultaneously analyzed for the quantity of amoxicillin and paracetamol in the medium – this also represents the number of unreacted drugs at each time amplitude and could be used as a measure of interaction between both drugs. Amoxicillin and paracetamol were determined at 210 and 290 nm respectively, being appropriate wavelengths on deconvolution of their overlain spectra.

Data Analysis: The data obtained in this study were analyzed using Microsoft Excel (2016).

RESULTS AND DISCUSSION

Determination of maximum absorption wavelength

Two distinctive peaks at 240 nm and 260 nm were observed for AMX, while for PCM at 290nm and 340 nm, with maxima at 240 nm and 340 nm for AMX and PCM (AMX+PCM) respectively in phosphate buffer (pH 6.8), as shown in Figure 1. There are overlaying spectra observed between amoxicillin and paracetamol spectra. The spectrum for the combined drugs (PCM +AMX) showed that it is addictive.

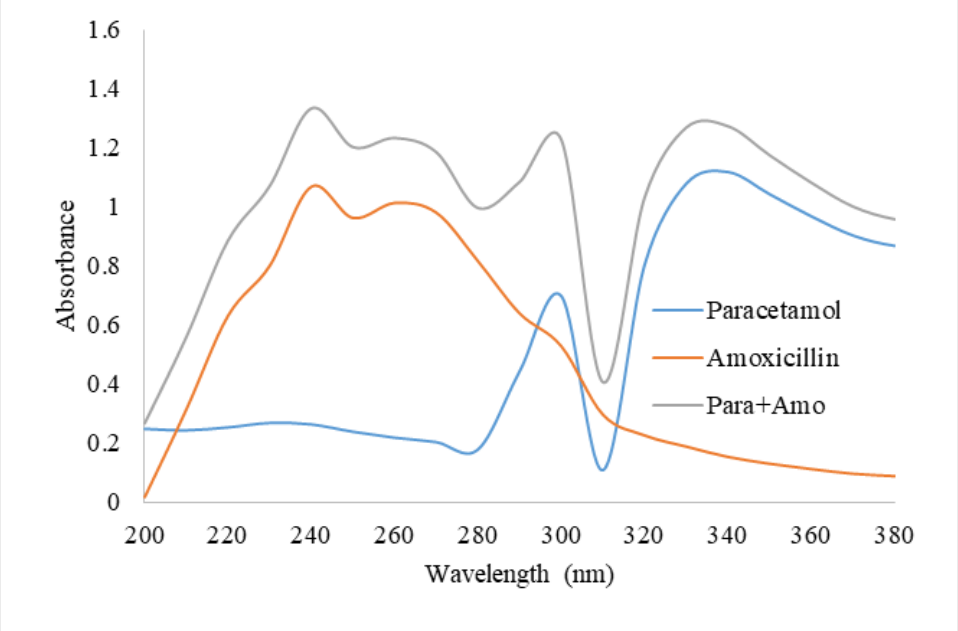

Figure 1: Zero Order Derivative Spectra of AMX, PCM, and AMX + PCM

Deconvolution of Spectra

The deconvolution of the overlain and underlain spectra in Figure 3, was by derivative spectroscopy. Figure 2 shows the 1st – 4th order derivative for amoxicillin and paracetamol. The 3rd order was adjudged most appropriate - with the AMX showing absorption at 210 nm and PCM at the zero-line crossing point, while the reverse is the case for PCM and AMX with absorption at 290 nm and zero-line crossing point respectively.

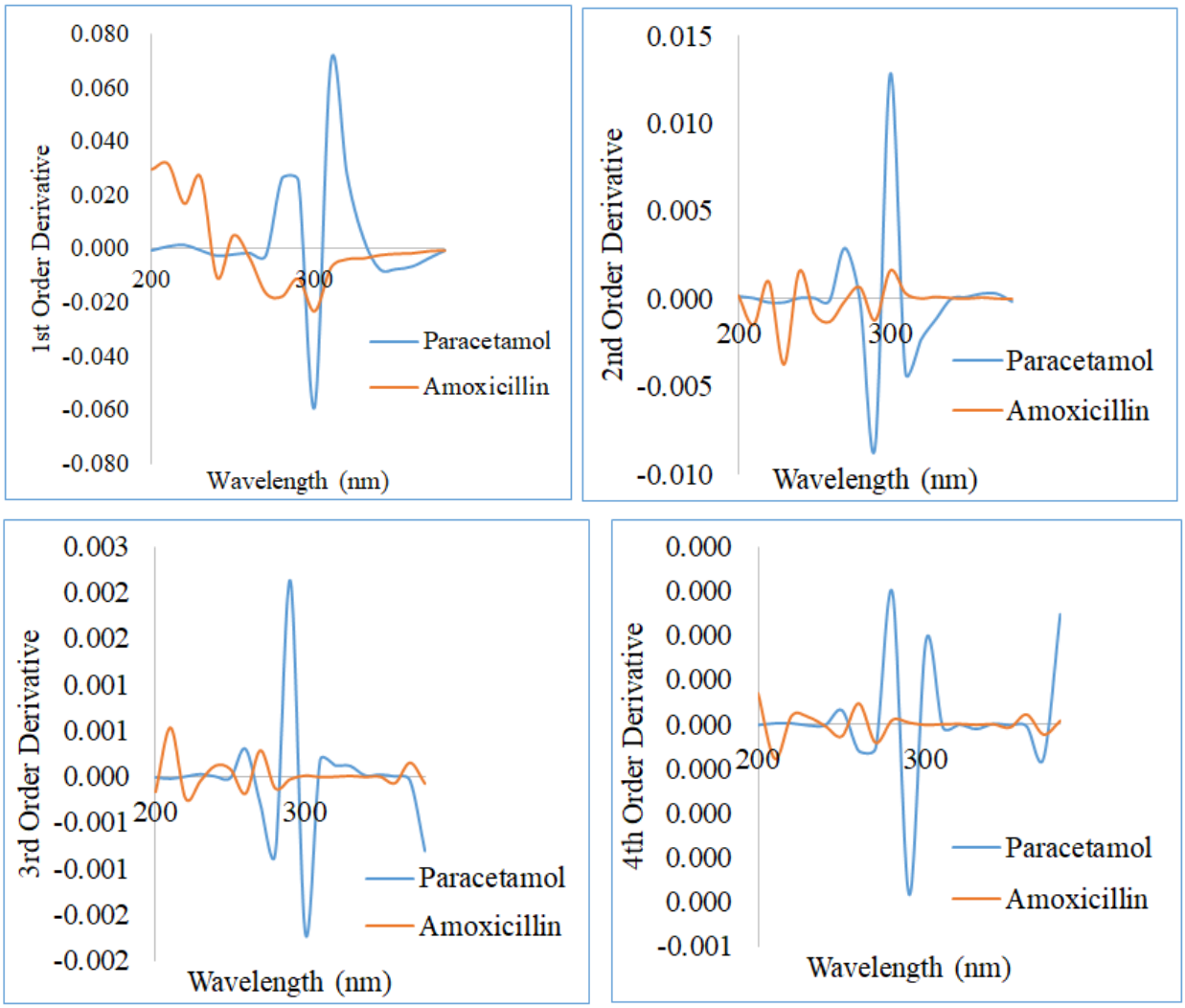

Figure 2: 1st – 4th Order Derivative Spectra of AMX and PCM

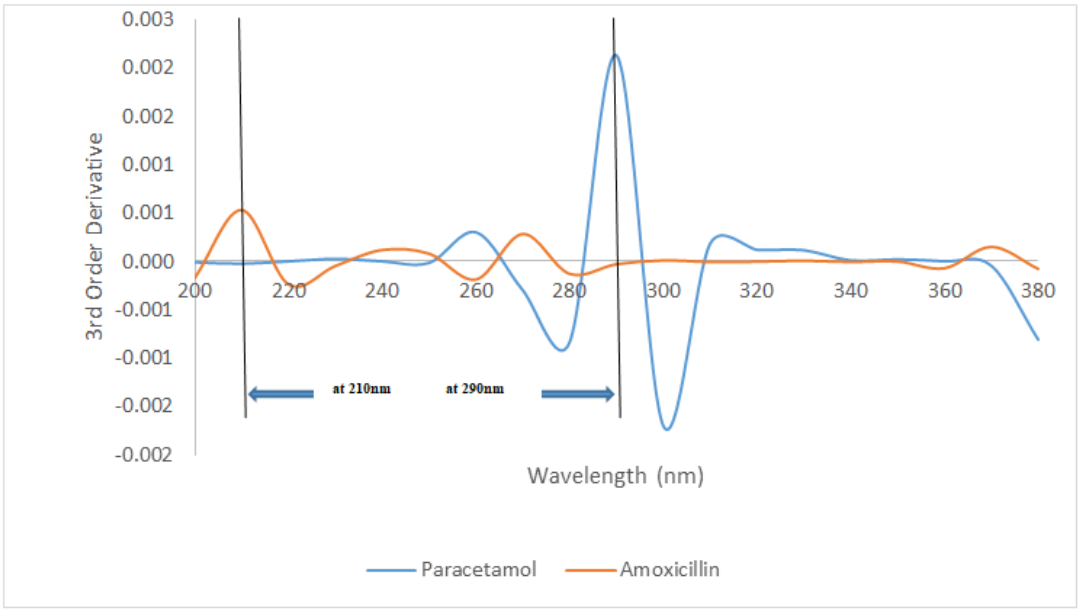

Figure 3: 3rd Order Derivative spectra for AMX and PCM

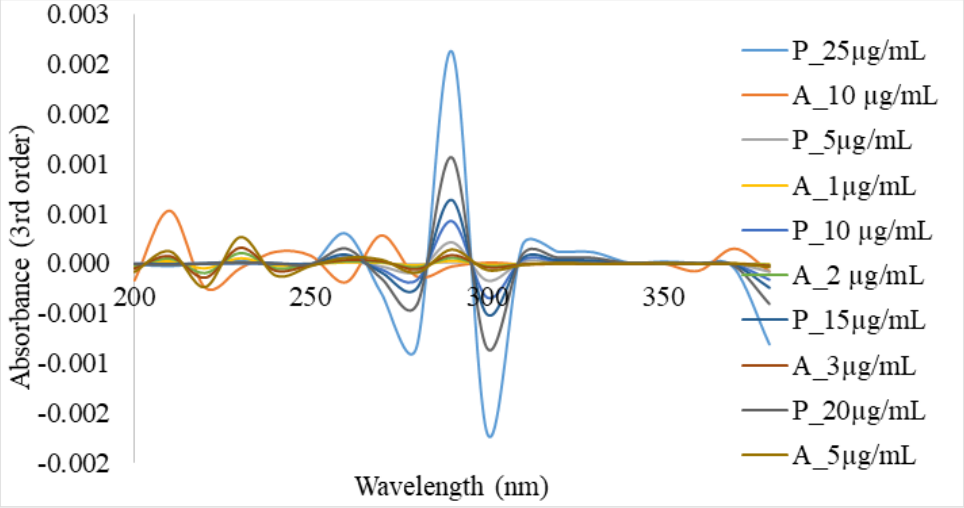

Figure 4: 3rd Order Derivative spectra for AMX (1, 2, 3, 5, and 10 µg mL^{-1}) and PCM (5, 10, 15, 20, 25 µg mL^{-1}) at various concentrations

Analytical Performance

The optimum conditions obtained by the assay methods for AMX and PCM in a phosphate buffer medium (pH 6.8) are presented in Table 1. The calibration curves for AMX and PCM standards showed good linearity at concentrations ranging from 2 – 10 µg mL^{-1} and 5 – 25 µg mL^{-1} respectively, with corresponding correlation coefficients (R) being 0.999 and 0.998. The intercept and slope for AMX were -0.0148 and 0.0732 respectively, while for PCM values were 0.0071 and 0.148 (n =5). The LOD and LOQ were 0.16 µg mL^{-1} and 0.59 µg mL^{-1} for AMX respectively while PCM had corresponding values of 1.48 µg mL^{-1} and 4.5 µg mL^{-1} (where LOD = $3.3S_a/y$; LOD = $10S_a/y$; S_a is the SD of the intercept or regression line and y is the slope). These aforementioned values depicted good reliability and repeatability of method.

Table 1: Optimum conditions for AMX and PCM assay in phosphate buffer (pH 6.8)

Parameter	AMX	PCM
Wavelength (nm)		
- Zero order derivative (nm)	240, 260	290, 340
- 3rd order derivative (nm)	210 (zero line crossing PCM)	290 nm (zero-line crossing AMX)
Beer's conc. Range (µg mL^{-1})	2 - 10	5 - 25
Limit of detection (LOD) (µg mL^{-1})	0.16	1.48
Limit of quantification (LOQ) (µg mL^{-1})	0.59	4.5
Regression equation		
Slope	0.0732	0.0146
Intercept	-0.0148	0.0071
Correlation coefficient (R)	0.999	0.998

Table 2: Assay of different brands of Paracetamol

Sample ID	Label claim (mg/tablet)	Amt found ± Sd (mg/tablet)	%RSD	SEM	Drug Content (%)
Paracetamol	500	492.84 ± 1.98	0.40	1.18	98.40 ± 0.44
Amoxicillin	500	481.22 ± 1.59	0.32	0.94	96.04 ± 0.29

Disintegration Time

The disintegration time for AMX capsules and PCM tablets they were ranged from 5 – 8 minutes for both drug formulations that were carried out separately and jointly. They complied with the USP/BP specification for the disintegration test [34, 35]. This biopharmaceutical test for solid dosage form is key in evaluating drug release and could be used as a gauge to indicate the lack of (or presence of) batch uniformity and inconsistency in solid dosage formulations [36]. The content of PCM and AMX in the formulations was 98.40 ± 0.44 % and 96.04 ± 0.29% respectively. These values were considered satisfactory and complied with the acceptable limits by the BP [34]. The %RSD and SEM values for both drugs were low and this implied good sensitivity.

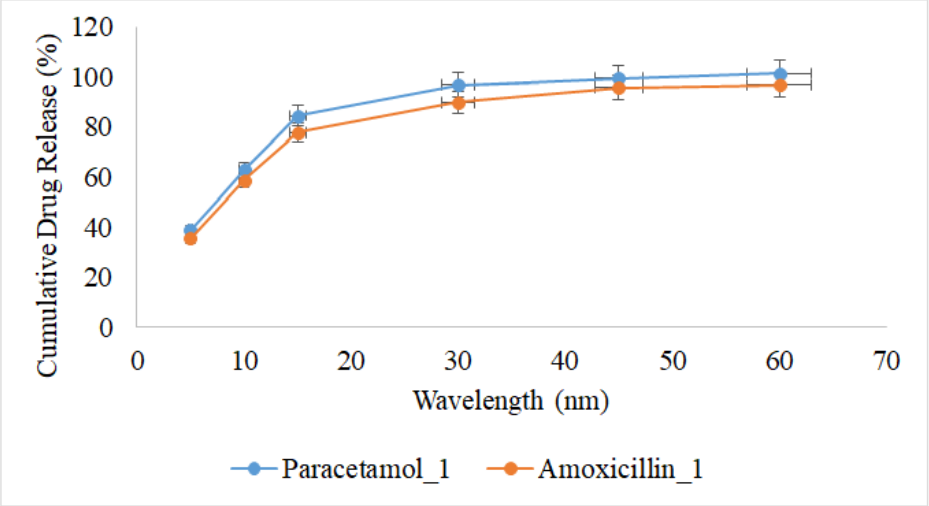

Figure 5: Dissolution profiles of PCM and AMX from individual release experiment

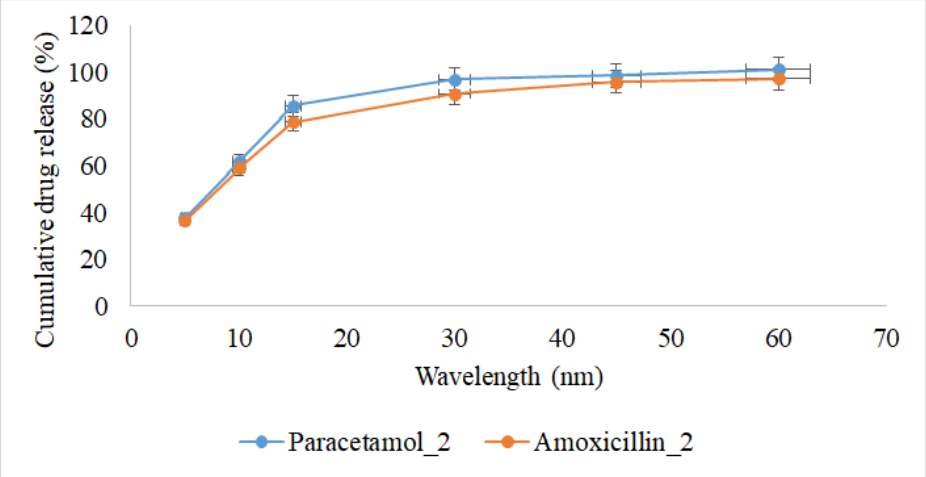

Figure 6: Dissolution profiles of PCM and AMX from joint-release/interaction experiment

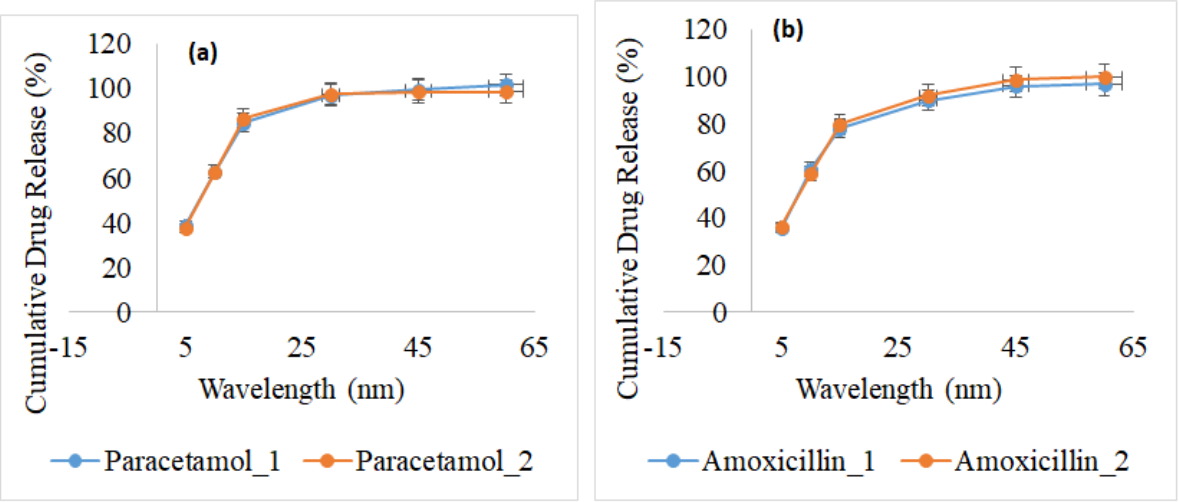

Figure 7: Comparative dissolution profile of (a) PCM from individual release (PCM _1) and joint-release (PCM _2) (b) AMX from individual release (AMX _1) and joint-release (AMX _2)

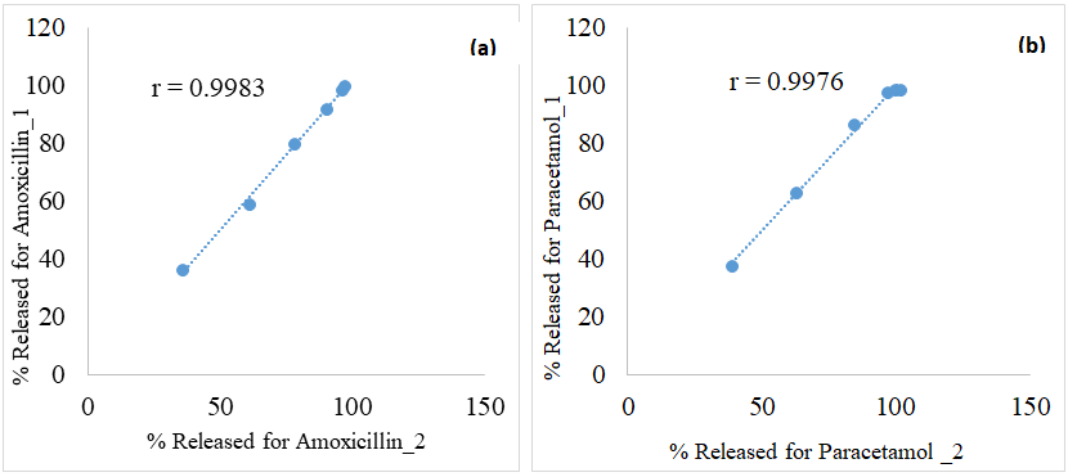

Figure 8: Comparative *in-vitro* release plot during individual and joint studies for (a) AMX and (b) PCM through t_5 –t_{60}

In-vitro Drug-Drug interaction studies

The drug dissolution profile is an indicator of how an ingested medication is released into the body fluids for absorption and availability in the circulatory system for therapeutic effectiveness. Also, the amount of drug released, absorbed, and available could be affected when there is drug-drug interaction if two or more medications are ingested at the same time. Figures 5 – 7, capture results obtained in applying the two-phase experimental design on *in-vitro* drug-drug interaction studies.

Figure 5 shows the *in-vitro* dissolution profile for AMX and PCM for individual drug release experiments. Both drugs complied with the stipulated BP/USP rate of 80% or more of actives within 30 minutes (t_{30}) of the dissolution experiment [34, 35]. At t_{60}, 101.50% PCM and 96.83 % AMX (of label claim) were released into the buffer medium at pH 6.8 (Table 2). The joint-drug-dissolution studies for AMX and PCM interaction (i.e., drug-drug interaction) are presented in Figure 6. The results for this study were obtained by derivative spectroscopy, with the zero-order UV spectroscopy of both drugs showing overlaying/underlaying of their UV spectra (Figure 1). The *in-vitro* interaction between both drugs was assessed using the 3rd Order Derivative of AMX and PCM UV spectra (Figure 2, Figure 4). Measurements for AMX and PCM were done at maxima amplitudes of 210 nm and 290 nm respectively – these wavelengths also corresponded to the zero-crossing points for AMX and PCM (Figure 3). This shows that AMX recorded zero absorbance while PCM showed a significant absorbance and vice versa. Therefore, Figure 6 – the *in-vitro* drug dissolution profile is adjudged akin to Figure 5 and portrays no significant drug-drug interaction between AMX and PCM. In addition, the comparative dissolution profiles for AMX and PCM indicated no interaction in the joint-release studies (Figure 7). The Pearson correlation coefficient (r) from regression plot from individual-release (AMX _1, PCM _1) against joint-release (AMX _1, PCM _2) for AMX and PCM were 0.9983 and 0.9976 respectively (Figure 8, Table 1). These values signify the very strong relationship between the number of drugs released during individual and joint studies for AMX and Paracetamol and could be stated that there is no interaction between both drugs.

CONCLUSION

The application of a drug dissolution experiment to simulate the potential interaction of orally administered AMX capsules and PCM tablets - that subsequently would lead to their release, absorption, and bioavailability for therapeutic activity was successfully designed and conducted. There was no AMX-PCM interaction in the phosphate buffer (pH 6.8) test medium, used to mimic a normal physiological environment for the individual and joint *in-vitro* qualitative analysis. Also, no significant difference was observed in the *in-vitro* drug release profiles for AMX and PCM, while comparative profiles were observed to have a very strong correlation between individual-release and joint-release profiles for AMX_1, AMX_2, and PCM _1, PCM _2, with r values being 0.9983 and 0.9976 respectively. Hence, no potential drug-drug interaction of the two candidate drugs was observed in the study.

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