Scientific African 19 (2023) e01537

Contents lists available at ScienceDirect

Scientific African

journal homepage: www.elsevier.com/locate/sciaf

Comparative evaluation of acetaminophen form (I) in commercialized paracetamol brands

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ARTICLE INFO

Article history:
Received 10 February 2022
Revised 23 December 2022
Accepted 27 December 2022

Editor: DR B Gyampoh

Keywords:
Paracetamol
Excipients
PCA
Raman / FT-IR spectroscopy
XRD
Drug release

ABSTRACT

The vibrational spectroscopy (FT-IR/Raman) and X-ray diffraction techniques are combined alongside the principal component analysis (PCA) as novel integrated analytical techniques to comparatively investigate latent chemical information and quality discrepancies regarding twelve (12) commercialized paracetamol (APAP) brands. This research aim is to present an advanced computational screening approach using spectroscopic and X-ray diffraction techniques with PCA as a tool to investigate the structural properties of pharmaceutical solid drugs by vibrational mode and diffraction pattern analyzes. Herein, the acquired vibrational, absorption, and diffraction datasets of APAP functionalities were collected at spectra and diffraction regions of intense peaks to develop predictive PCA models. Interestingly, the PCA models invalidate drug falsification in all the brands and predicted dissimilarities arising from observed differences in the vibrational/absorption modes of APAP form (I) in some brands due to excessive use of cheap (fillers and hydrocolloid alternatives) excipients. The PCA-PXRD model unveils discrepancies regarding the contrasting diffraction patterns (structure-property relationships) observed for APAP form (I) in the brands, which suggests differences in their pharmacokinetic properties cause an unapparent structural modification. Nevertheless, the comparative drug release studies present a%CDR between 93 and 98% in 30 min for all the brands, thus, structural modifications of APAP form (I) as observed in some brands show no serious effects on the%CDR and/ or solubility. Finally, it is expected that this work will contribute to the advances in screening techniques toward addressing the global drug challenges, especially in developing countries.

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https://doi.org/10.1016/j.sciaf.2022.e01537

Introduction

According to the United State Department of Food and Drug Administration (FDA), World Health Organization (WHO), and other drug control agencies, drug manufacturing processes must be confined within the pharmacopeia regulations and standards for medicinal safety, therapeutic potency, efficacy, security regulations, stability of the active pharmaceutical ingredient (API) and quality consistency to mention a few [1,2]. Poor formulation standards resulting from the use and deliberate overuse of cheap/substandard excipients like cheap hydrocolloids alternatives (carbohydrate-based fillers/gliders), and cases of API adulteration in commercialized drugs are the major challenges of drug formulation in a developing country. Unfortunately, the flexibility of governmental policies on local manufacturers of generic drugs and the marketing of such drugs are directly contributing to health issues and serious insecurity [3].

Important physical properties like solubility, dissolution rate, and API stability contribute significantly to the therapeutic effectiveness of a drug in the final tablet product (oral administration) for the desired permeability and oral bioavailability. These parameters and other issues like disintegration, tablet hardness, dissolution, and flow properties are mostly readdressed during formulation processes mechanically/traditionally on a molecular level or through the use of excipients (inactive ingredients) such as solubilizes, disintegrants, emulsifying, and stabilizing agents [4]. Although most excipients are regarded as inactive materials, nevertheless some have functionalities that present the possibility of interacting and transforming the physicochemical properties of an API in final product form. Crystalline or as amorphous materials, most excipients such as calcium hydrogen phosphate, sorbitol, magnesium stearate, mannitol, and sucrose exhibit the tendency to trigger a polymorphic transformation of the API, a phenomenon that limits or modifies the therapeutic/biological performance of the drug [5]. Proper excipients selection and judicious handling/usage during manufacturing processes are important to negate formulation issues like polymorphism and form transformation. Likewise, the subsequent changes in the pharmacological and pharmacokinetic profile of an API that is supposed to be maintained throughout the production processes [6,7].

Structural modification (polymorphism) of an API is a common phenomenon that occurs due to poor production practices, especially when profit maximization is prioritized over drug quality. Reported evidence of poor production practices using cheaper alternatives of APIs/excipients in drug formulation is linked to local pharmaceutical industries in developing countries, where reports on trademarks infringements to produce packages and sell falsified medicines (substituted formulations/substandard qualities) are rampant [8]. In addition, the practice of profit maximization through the use of substandard APIs, excipients, and related alternatives is predominant and results in the production/marketing of harmful drugs with entirely modified biological/therapeutic effectiveness (efficacy) in many cases [9,10].

Acetaminophen or N-acetyl-para-aminophenol (APAP), or Paracetamol is an analgesic and antipyretic drug with no significant anti-inflammatory activity, and its commercialized generic brands (tablets) are one of the easily purchased Over-The-Counter (OTC) drugs, poisonous or toxic on excessive use, while additional complications resulting into liver and kidney damage, skin reactions and asthma [11,12]. Paracetamol drugs are sometimes in formulation with non-steroidal anti-inflammatory drugs (NSAID) like ibuprofen, aspirin, and caffeine, in known proportions (5–15% w/w) to synergize its antipyretic activity through combined therapy [13]. However, research studies on APAP forms and especially the comparative stability studies between the pharmaceutically preferred monoclinic form (I) and the metastable orthorhombic form (II) in formulation processes, and the details on phase transformation from form (II) to the stable form (I) during formulations indicate similar bioavailability and clinical safety on precautions [14,15].

The vibrational spectroscopic techniques (Raman and FT-IR) are sensitive analytical tools that provide quick material characterization and identification of materials' chemical and structural properties. Thus, provide high-resolution spectra information from in situ probes, offline, in-/at-/, and online interactions for quantitative and qualitative data analysis [16]. Raman spectroscopy is a non-destructive technique that generates molecular information via an inelastic scattering phenomenon when the molecular system interacts with a photon of energy at a specific frequency. The FT-IR spectroscopy generates vibrational transition that corresponds to changes in molecular dipole moments and results in the absorption/transmission of infrared photons, the photon's energy, therefore corresponding to the energy differences between the vibrational states. These spectroscopy techniques are complementary "green analytical" techniques and are widely employed for real-time quantitative and qualitative analysis, especially for pharmaceutical-related materials [17–19].

Applications of vibrational spectroscopic techniques with chemometrics tools continue to gain potential significance and prominence as reliable techniques/approaches for solving complex scientific challenges. Although each spectroscopy technique has unique analytical potential and correctness, interesting reports on the combination with chemometrics tools such as principal component analysis (PCA) for unveiling chemical information from complex spectroscopic data are predominant to individual spectroscopic techniques [20–22]. PCA uses an orthogonal transformation to decompose a dataset and generate linearly uncorrelated variables called the principal components (PCs) that present maximized variance of trends (dominant patterns) within the dataset. However, its combination with spectroscopic techniques enables efficient extraction of correlated observations/information from datasets i.e. a visual projection of score and loading plots for predictive analytics [23–25]. However, a few reports are available in the literature on the combination of spectroscopic techniques with Chemometrics tools for detecting polymorphism in APIs, analysis of impurities in drugs, detecting adulterants and counterfeit drugs, and drug characterization [26–29]. More so, only a few reports demonstrated the combination of analytical techniques, chemometrics methods, and pharmacotechnical studies as an advanced screening approach for investigating the structure-property and quality of pharmaceutical solid drugs.

X-ray diffraction is a molecular structure identification technique that presents diffraction patterns unique to specific crystalline solid material in interaction with X-ray radiation. The diffraction pattern for a pure sample is considered the fingerprint identity of its atomic arrangement, while the integrated intensities of its diffraction peaks are considered to be proportional to its homogeneity or amount of components. Therefore, the combination of XRD with PCA continues to gain prominence in the qualitative and quantitative assays on structural identification of polymorphic phases in solid materials, identification of novel structures, monitoring stances of polymorphic transformation in finished drug formulations and detecting traces of adulterants in drugs [30–32]. The combination of chemometrics tools with analytical techniques to decompose diffraction datasets into a visual predictive model for analysis of latent trends is potentially gaining more attention in the context of material sciences [33–35].

This article aims to demonstrate the advantages of combining vibrational spectroscopic (FT-IR/Raman) and XRD techniques with PCA as potential analytical tools to comparatively investigate drug falsification, deliberate use/overuse of cheaper APAP form (I) and excipients, and especially the effects of excessive use of cheap hydrocolloids alternatives (carbohydrate-based fillers/gliders) in 12 paracetamol brands (tablets). To achieve reasonable analytical models and predictions, better acquisition and collections of desired FT-IR/Raman spectra and X-ray diffractions of the brands, pure APAP form (I) and prepared paracetamol tablets of varied APAP/excipients concentrations are prioritized for developing reasonable analytical models. The Raman spectra region 200–1650 cm^{-1} predominant of important vibrational modes of APAP with intense peaks of bonded functionalities like the aryl CH (para sub) at 857.2 cm^{-1}, CNH stretching at 796.9 cm^{-1}, ring breathing at 857.4 cm^{-1}, OH substituted stretching at 1236.2 cm^{-1}, CH aryl bend at 1168 cm^{-1} was selected. Likewise, the IR absorption modes of APAP functionalities in the spectra region 1800–1300 cm^{-1} where the C=O stretch, C=C stretch, O–H, and N–H bends present intense peaks were accessed and pre-processed for developing predictive PCA models. The X-ray diffraction data selected from the range of 5°–40° (2θ) of the brands were used to develop a PCA-XRD model that investigates diffraction patterns and unveils discrepancies regarding structural modifications of the APAP form (I) in the brands. Finally, dissolution analysis of the brand and designed tablets was carried out at standard experimental conditions (900 mL of deionized water, at 37.5 °C and 60 amp) to investigate the effects of structural modification and quality on their respective drug release. Finally, this study is expected to present new knowledge on the combination of analytical tools/ techniques as an advanced computational screening approach to monitoring the qualities of pharmaceutical solid drugs.

Materials and methods

Materials

Acetaminophen (APAP) and Caffeine (CAF) were purchased from Sigma Aldrich at a purity greater than 99% (Analytical grade). Excipients; croscarmellose sodium, magnesium stearate, hydroxypropyl cellulose, crospovidone, microcrystalline cellulose, sodium starch glycolate, corn starch, and hydroxypropyl methylcellulose were purchased from Medley pharmaceutical industry, Sao Paulo State, Brazil. Ten (10) sachet samples of four (4) Paracetamol brands; Tylenol® (500 mg APAP and 65 mg CAF), Prati® and Tyflen® both contained 500 mg APAP, and Teuto® 750 mg APAP, were purchased at different drug stores in São Paulo city. Likewise, Ten (10) sachet samples of eight (8) paracetamol brands were purchased at registered drug stores in Ibadan and Lagos cities (Nigeria) and denoted gskEx that contained 500 mg APAP and 30 mg CAF, while Emc, Ppf, Tum, MB, Emz, DrM, and Pgsk all contained 500 mg APAP. The Raman spectroscopy analysis and powder XRD analyzes of the commercial paracetamol tablets affirmed APAP form I as the active ingredient. The tablets are indicated to contain acetaminophen within 100 ± 10% of the labeled claim and were of the same manufacturing year.

Paracetamol trihydrate synthesis

The methods and conditions for preparation of trihydrate of paracetamol follow as reported by McGregor et al. which was synthesized by slow cooling of an aqueous solution of paracetamol to 0 °C [36].

Raman spectroscopy

Raman spectra of all samples were acquired/collected using a B&W Tek BWS 415785H Raman spectrometer coupled to a microscope B&W Tek BAC 151B This device has an excitation laser of wavelength 785 nm with a spectral resolution of 3.5 cm^{-1}, 120 s of acquisition time, spectral range 200–2180 cm^{-1}, laser power of 70 mW and is managed by BWSpec4.03 software.

Fourier transform infrared spectrophotometry (FT-IR)

Screening and characterization were performed on a Shimadzu IRprestige-21 FT-IR using the potassium bromide (KBr) pellet technique. FT-IR Spectra were acquired using 64 scans, a resolution of 4 cm^{-1}, between wavenumber ranging from mid-infrared region 4000–400 cm^{-1}. Like Raman, FT-IR spectroscopy provides relevant chemical information about the fundamental vibrational and rotational modes of functionalities in molecules.

Powder X-ray diffraction (PXRD)

The X-ray powder diffraction analysis of partially milled samples was performed on a Multiflex X-ray diffractometer (Rigaku Corporation, Tokyo, Japan), using a copper (Cu) kα radiation (1.54 Å) at a voltage of 40 kV and current of 30 mA. The samples were carefully milled and scanned from 5° to 40° (2θ), at a step size of 0.2° θ/minute under a scan rate of 2°/min to provide structural information, crystallinity level, and the possibility of polymorphism in the brands. However, chemical and mechanical contaminations were prevented, and the samples were moderately milled before the PXRD analyzes to achieve good diffractions.

Statistical tools

All the chemometrics computations were carried out using Matlab R2011a (The Mathworks, Inc., Natick, MA, USA) and the PLS Toolbox 52 (Eigenvector Research, Inc., Manson, WA, USA). OriginLab software Pro9 and Excel software are used to plot/organize raw Raman/FT-IR spectroscopy and X-ray diffraction data into the Matlab R2011a /PLS toolbox.

Principal component analysis (data preprocessing)

The PCA function in the PLS toolbox 52 and Matlab R2011a software was employed for decomposing and transforming data into orthogonal factors (principal components, PCs) after different data pre-treatment methods to achieve satisfactory discriminations/correlations between samples. Raman spectra data (200–2180 cm^{-1}) was loaded; regions 600–900 cm^{-1} and 1000–1400 cm^{-1}, were selected and pre-processed using Savitzky-Golay first derivative, Normalization, and mean centering. FT-IR spectra data (4000–400 cm^{-1}) was loaded into the PLS toolbox and wavenumber region 1800–1400 cm^{-1} was selected for pre-processing using first derivative (polynomial order: 2, filter width: 21), Normalization (2-Normal, length 1), and mean centering. The selected Raman/FT-IR spectra regions for PCA models represent the region of strong characteristic peaks related to the vibrational modes of APAP (Form I). Likewise, diffraction data taken from 5° to 45° (2θ) was pre-processed using the first derivative (polynomial order: 2, filter width: 31), Normalization (2-Normal, length 1), and mean centering. The pre-processing methods remove extraneous sources of variations in the diffraction data, thus linearizing the response and result into a fit PCA model that presents optimized variance of interest.

Dissolution studies

A dissolution study for the paracetamol tablets was carried out in 900 mL of deionized water at a set temperature of 37 $\pm$ 0.5 °C, at a rotating speed of 60 rmp to observe the effects of diffraction variations on drug release using USP type II apparatus (Electrolab, Mumbai, India). Samples of the twelve paracetamol brands and the prepared paracetamol sample (400/500 mg) were introduced into the dissolution media, aliquot (2 mL) were withdrawn (replace with deionized water) at designed time intervals and assayed for their APAP content using UV-vis spectrophotometer at 234 nm, and performed in 3 replicas. The average of the experiments and the employed sink conditions was used to calculate the percentage of drug dissolved from calibrated Beer's plots. The comparative drugs released between 5 and 50 min were determined to predict the effects of structural modification on the dissolution rate.

Sample tablets preparation

According to Table 1, an experimental design is used in line with pharmaceutical standards to prepare various compositions of uncoated paracetamol tablets. Herein, using APAP form (I): between 17.54% w/w (100.00 mg) to 87.72% w/w (500.00 mg), hydrocolloids excipients: between 7.02% w/w (40.00 mg) to 76.32% w/w (435 mg), and excipients to weigh approximately 570 mg/tablet. The sample mixing was achieved using a tridimensional mixer (Turbula® T2F) at 40 rpm for 50 min, and direct compression into flat-surfaced tablets was done using a TDP-5 desktop table. The compression force of

Table 1
Composition and amount of APAP and excipients used in modeled samples (A-F).

COMPOSITION (mg)	PREPARED PARACETAMOL SAMPLE TABLETS					
	A	B	C	D	E	F
N-acetyl-para-aminophenol (APAP)	500	400	350	300	200	100
Starch related materials	20	55	85	95	125	185
Cellulose related materials	20	70	100	140	210	250
Colloidal silica dioxide	10	10	10	10	10	10
Sodium croscarmellose	10	10	10	10	10	10
Magnesium stearate	10	10	10	10	10	10
HPMC	–	5	5	5	5	5
Total weight	570	570	570	570	570	570

10 kN (100 MPa) was applied for 30 s to the well-mixed sample to achieve compact tablets that were used for production quality prediction and dissolution rate analysis.

Results and discussion

Vibrational spectroscopic (Raman/FT-IR) techniques

The vibrational spectroscopy techniques are associated with molecular functional modes and provide complex chemical information over a different and limited frequency range that depends on the experimental processes and magnitude of the associated energy differences. The applications of Raman/FT-IR spectroscopic techniques for characterization, material authenticity, and quality control measures not only demonstrate its potential and significance but also present the limitations of each technique when analyzing and/or interpreting similar but complex spectra. Nevertheless, spectroscopic techniques continue to demonstrate high analytical potential when combined with a chemometrics technique like PCA for qualitative and quantitative analyzes and especially for visual analysis of component evaluation, identification, and classification. Herein, Raman and FT-IR spectra analyzes of the 12 paracetamol brands analyzed by PCA at selected spectra regions are shown in Fig. 1a,b, and these 2D score plots indicate comparative differences in the vibrational and absorption modes based on the principal ingredients /materials present in each brand.

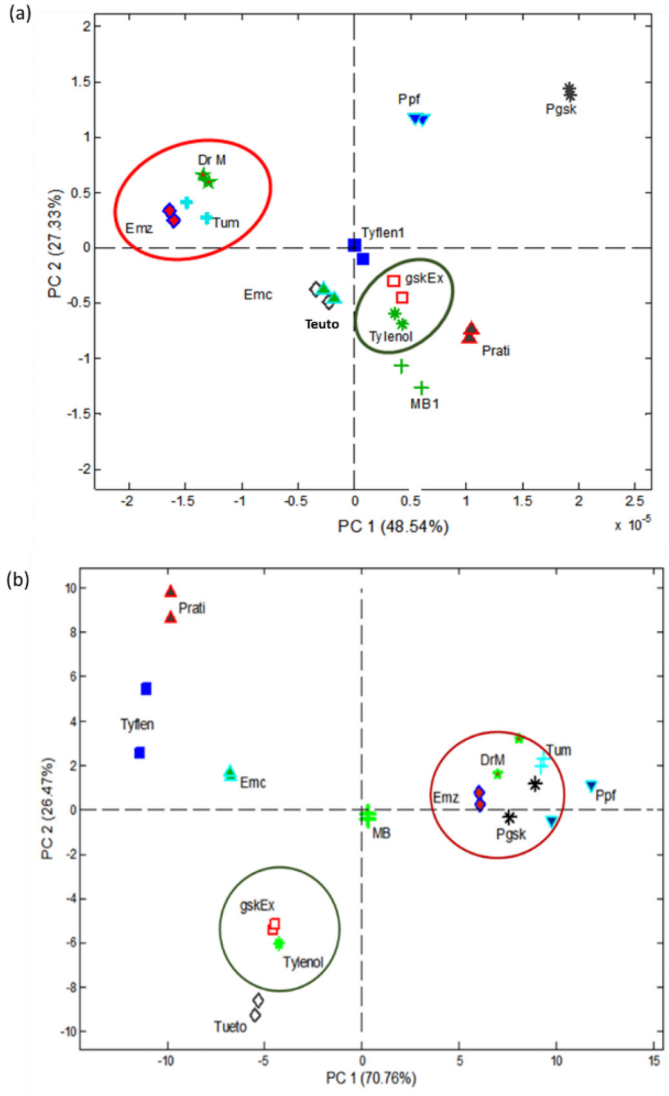

Fig. 1. 2D comparison of paracetamol brands; (a) Raman -PCA model, and (b) FT-IR PCA model.

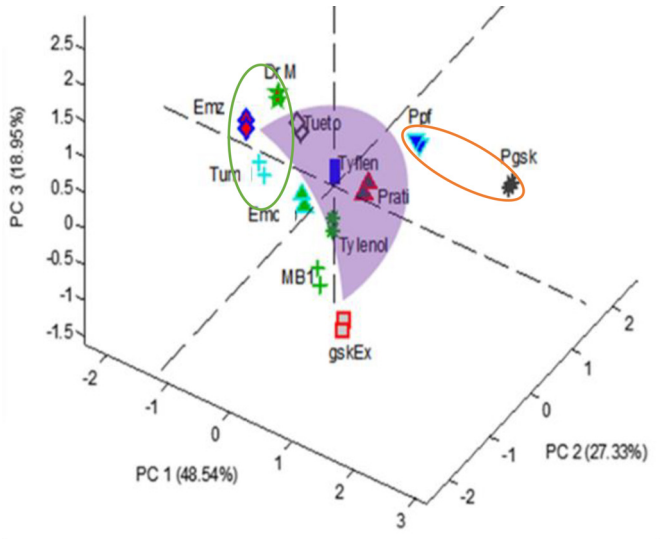

Fig. 2. Raman-PCA 3D model of the paracetamol brands.

Through visual inspections, limited information is obtained from 240 acquired spectra of 12 paracetamol brands with identical vibrational modes assigned to the functional groups in pure APAP form (I). Herein, the spectra were modeled to verify their quality differences, which depend on the equipment, capacity of each model, and result interpretations. Although, visual inspection of Raman and FT-IR spectra present no evidence of falsification, but presents limited information on the stability of the API, polymorphic transformation, and excipients effects, if present, remain hidden. Fig. 1a presents the Raman-PCA model with PC1 (48.54%) and PC2 (27.33%) indicating score plot correlation into three groups, and also evidence of discrimination between the brands as observed through their respective vibrational modes. This model correlates gskEx and Tylenol (green circle) which contain 35 and 65 mg of caffeine respectively, and indicates similarities between DrM, Emz, and Tum (red circle), while Ppf and Pgsk are projected considerably far away.

The FT-IR PCA model in Fig. 1b shows the PC1 (70.76%) and PC2 (26.47%) capture of absorption spectra variation for the paracetamol brands. The intense absorption modes of APAP functionalities at specific wavelengths present a complete understanding of the latent relationship between the brands. As expected, the PC1/2 correlate gskEx and Tylenol (green circle) based on their caffeine content, and correlate/group brands like Tum, Ppf, DrM, Emz, and Pgsk indicated with the red circle. Also, Prati, Tyflen, Emc, and MB are partially projected independently to the extreme from the middle of the model. Herein, the PCs show that certain factor is responsible for correlating Tum, DrM, and Emz, as indicated in the Raman-PCA model Fig. 1a. The models (Fig. 1a,b) show similar dataset features concerning using two-component PCA, and no significant information loss because score plot overload was avoided by observing a few scores from randomly selected spectra data.

Important chemical information about the functional groups and environmental chemical effects on each drug matrix at selected spectra region is observed through the interaction of Raman laser beam on heterogeneous paracetamol surface (±1 mm diameter). The 3D Raman-PCA model in Fig. 2 with a cumulative variance of more than 94% (PC1, PC2, and PC3), is the 3D representation of the model presented in Fig. 1a that will resolve observed trends and patterns in the models. Thus, an interesting correlation is observed between Teuto, Tyflen, Prati, and Tylenol, in the middle (shaded pink), this correlation was pondered and anticipated looking at the cluster in Fig. 1a, but hidden as observed with two PCs. The correlation in these brands occurred based on the uniformity of APAP vibrational modes like the CNH, aryl CC, and C=O stretches existing between the selected spectra ranges and may serve as evidence of APAP form I structure stability, modification, or/and effects of excipients load on the API. However, this model maintained the previously observed correlations between DrM, Emz, and Tum (green circle), and between Ppf and Pgsk (brown circle). Although, virtual and model analyzes of the paracetamol brands show no evidence of drug falsification or adulteration considering the sensitivity/accuracy of the models that detect Caffeine in Tylenol and gskEx. Nevertheless, these models indicate that certain factors correlate with DrM, Emz, and Tum, likewise, Ppf and Pgsk as projected within the models.

Fig. 3 presents a developed Raman-PCA models biplot of brands, APAP form (I), APAP hydrate, and starch (disintegrants) to investigate the effects of excess disintegrants and hydration on the vibrational modes and correlation. Herein, the model with PC1 (52.46%) and PC2 (26.89%) discriminates starch far away from the brands as an outlier, while it presents the previously observed correlation between DrM, Emz, and Tum (green circle) around APAP hydrate. The model indicates no strong correlation between the paracetamol brands and starch, even with the projection of Pgsk in the same quadrant. Although, this model shows low sensitivity to the analysis of starch, but indicates discrepancies regarding the vibrational modes of APAP form (I) in the brands like gskEx and Pgsk. The model in Fig. 3b presents PC1 (61.46%) and PC2 (17.83%) that capture more than 86% of cumulative variance that unveils the effects of hydration (APAP hydrate) on the brands. This

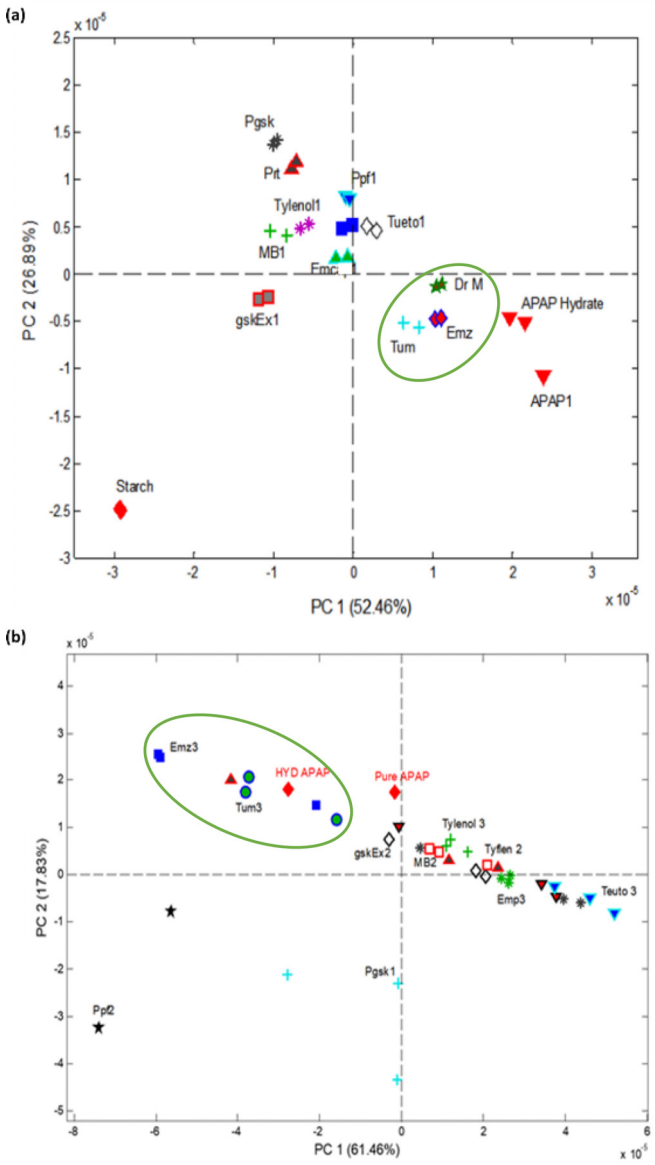

Fig. 3. Raman-PCA models: (a) effects of APAP hydrate and starch on brands, (b) APAP hydrate and Starch.

model project Ppf and Pgsk away from other brands, correlates Tum, DrM, and Emz with hydrate APAP, and away from Teuto, Prati, MB, Tyflen, and Tylenol. As observed, the 2D model indicates similar vibrational modes for Emz, Tum, DrM, and APAP hydrate, and as usual, it projects these brands away from Ppf and Pgsk.

Fig. 4(a–d) shows the PCA–FT-IR model analyzes of paracetamol brands to predict possible hydration or/and evidence of excess starch-related trends in the brands. Fig. 4a presents PC1 (83.42%) and PC2 (6.04%) with a cumulative explained variance of 89.46% that differentiates between the brands, pure APAP, and hydrated APAP based on selected absorption modes. The model is sensitive to correlating Tylenol and gskEx and indicates a relationship between DrM, Emz, and Tum, which according to the 3D model (Fig. 4b) are not strongly correlated to pure APAP. However, this 3D model with a cumulative variance of more than 93% projects Prati, Tyflen, and Teuto away from other brands, it correlates Ppf with Pgsk and indicates no evidence of hydration in the brands. It is worth mentioning that models developed herein show high sensitivity to each sample (brand) composition and excipient effects on vibrational and absorption modes of the API (APAP form I).

Fig. 4(c-d) presents the PCA−FT-IR model that investigates the presence and effects of excess disintegrants in the brands. The projection by PC1 (69.10%) and PC2 (20.74%) in Fig. 4c is a good example of incomplete principle component selection for the analysis of trends in a model. Here, the correlation between brands in black and red circles with starch is incomplete and misleading. However, the inclusion of PC3 (5.27%) to the model in Fig. 4d re-positioned (project) the aforementioned

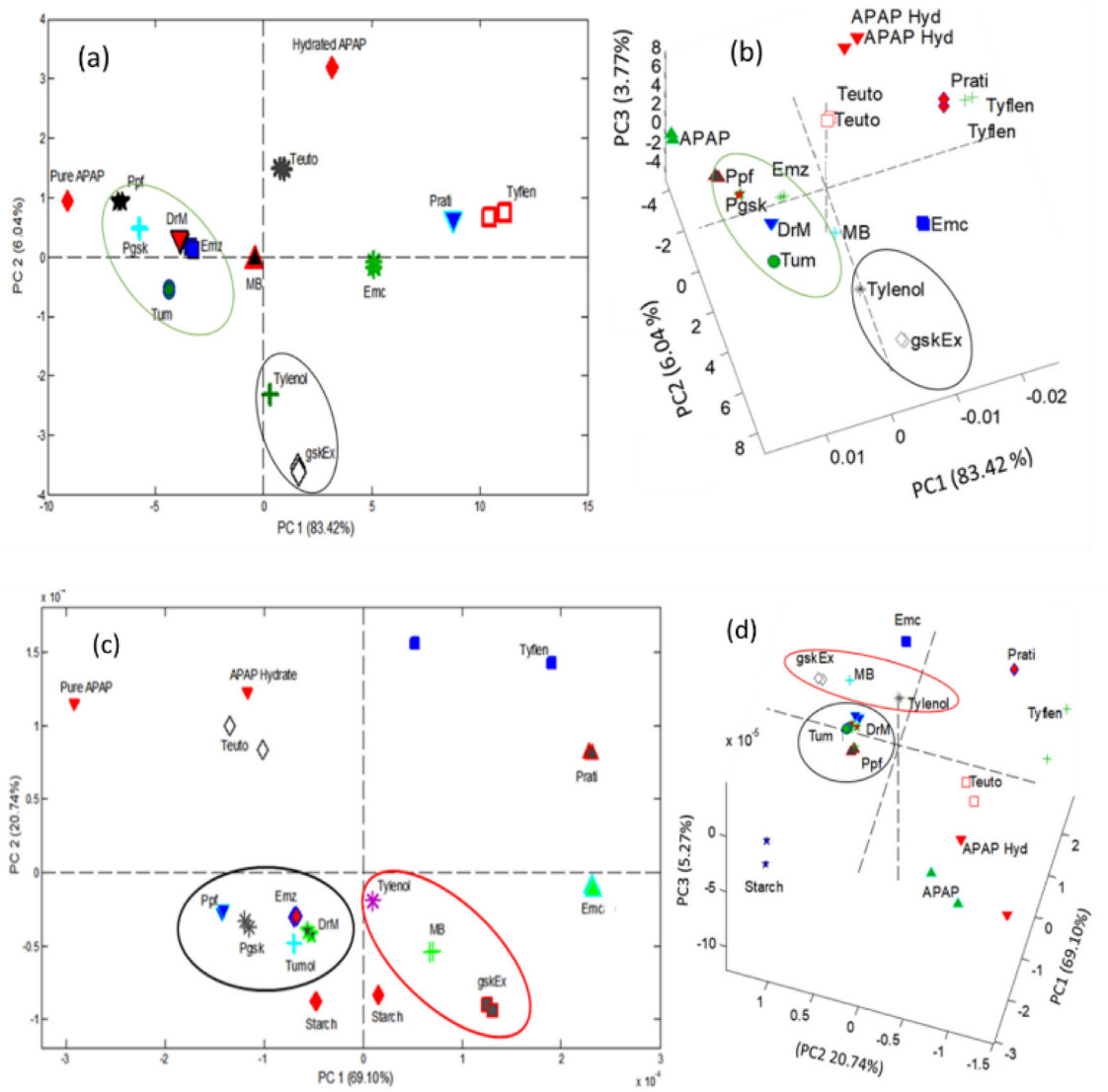

Fig. 4. PCA models of FT-IR spectra; (a-b) effects of APAP hydrate on the brands, and (c-d) effects of starch content analysis.

brands in the black and red circles far away from Starch. Although, this model with 95.11% cumulative variance shows no evidence of hydration or/and starch (disintegrants) over-use as observed, but indicates evidence of production dissimilarities in the brands.

Modeled tablet formulations vs. paracetamol brands

Table 1 shows the composition/amount of APAP and excipient in the modeled acetaminophen tablets (A-F) developed by direct compression to comparative investigate differences in the brands' formulation qualities (production dissimilarities). Fig. 5a presents a 3D PCA model of Raman spectra data of the modeled tablets vs. paracetamol brands showing the projection of samples with 100–200 mg/tablets, and the correlation of Tum, DrM, and Emz with the 400 mg/tablet. Here, MB, Pgsk, and Ppf are projected uniquely from the center of the model, and beside the 500 mg/tablet, thus indicating a good composition. All the brands indicate good production standards, as the model correlates Tyflen, Tueto, Tylenol, and Prati vertically in the direction of the 500 mg/tablet, and some brands beside it (MB, Pgsk, and Ppf), while others in-between the 400 and 500 mg/tablet sample. Although, the brand's correlation around the 500 mg/tablet sample is excellent, but not clustered as expected, thus indicating a significant difference in the vibrational modes of the brands.

The PCA–FT-IR analysis of modeled tablets vs. paracetamol brands in Fig. 5b indicates a correlation between Tum, DrM, and Emz (green circle) with the 400 mg/tablet at the center of the model, while the 500 mg/tablet aligns vertically with Tyflen, Tueto, Tylenol, and Prati (black circle) as observed in the Fig. 5a. The model is sensitive enough to discriminate 100,

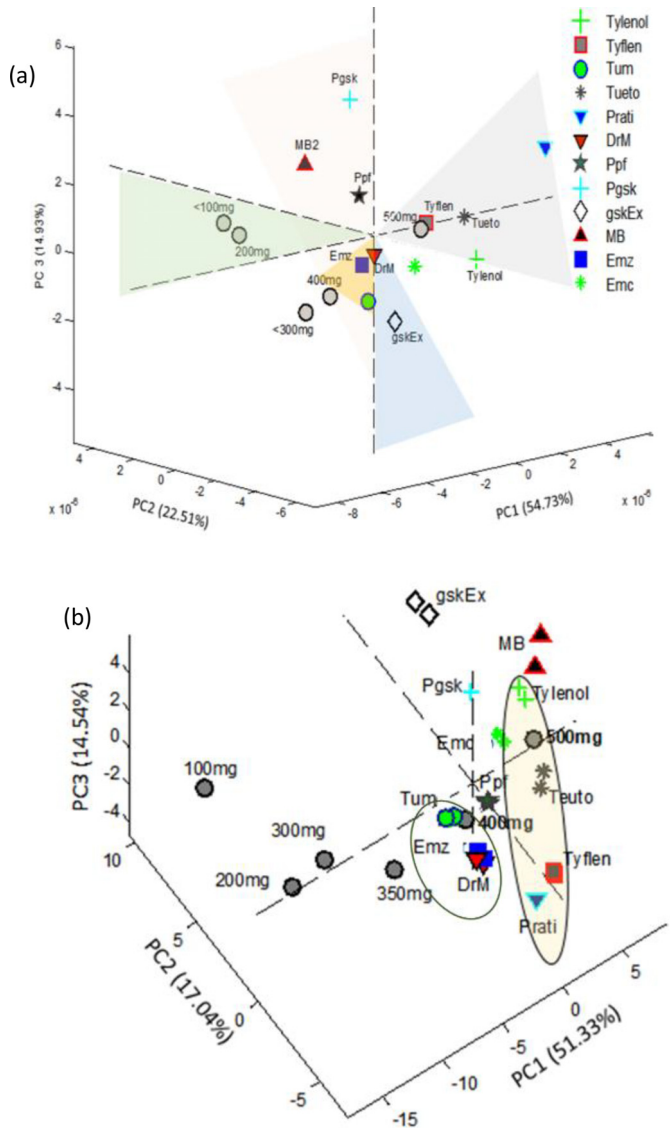

Fig. 5. 3D PCA analysis of modeled tablet formulations vs. paracetamol brands (a) Raman-PCA model and (b) FT-IR-PCA model.

200, and 300 mg/tablet samples away, and correlates the 400 and 500 mg/tablet samples with the brands. As observed in Fig. 5a, MB, Pgsk, and Ppf are uncorrelated and projected away from the vertical alignment of 500 mg/tablet and Tyflen, Tueto, Tylenol, and Prati. The developed spectroscopic models in this work indicate slight differences in the paracetamol brands and show that brands with similar APAP form (I) contents and excipient composition are mostly correlated. Interestingly, brands that are of the same manufacturer such as Ppf, Pgsk, and gskEx showed similar properties (trends) that are predicted by the model, and the brands show an important correlation concerning their brand quality and superiority (prices). Likewise, the models indicate differences in the qualities of the API (APAP form I) used in the formulation of some brands.

Powder X-ray diffraction analysis

The developed PCA models using the vibrational modes of the 12 paracetamol brand indicated major formulation similarities with no evidence of drug falsifications, and indicate differences in the level of crystallinity and expected variation in pharmacokinetic properties. The observed differences in the diffraction patterns (peak positions/intensities) of the brands compared to APAP form (I) are observed at 12.06, 13.82, 15.55, 16.74, 18.18, 20.39, 20.78, 23.49, 24.38, 26.55, 27.18, 29.29, 332.71, 36.22 and 38.5 ($2°\theta$), and these observations will arguably result from manufacturing processes (formulation quality).

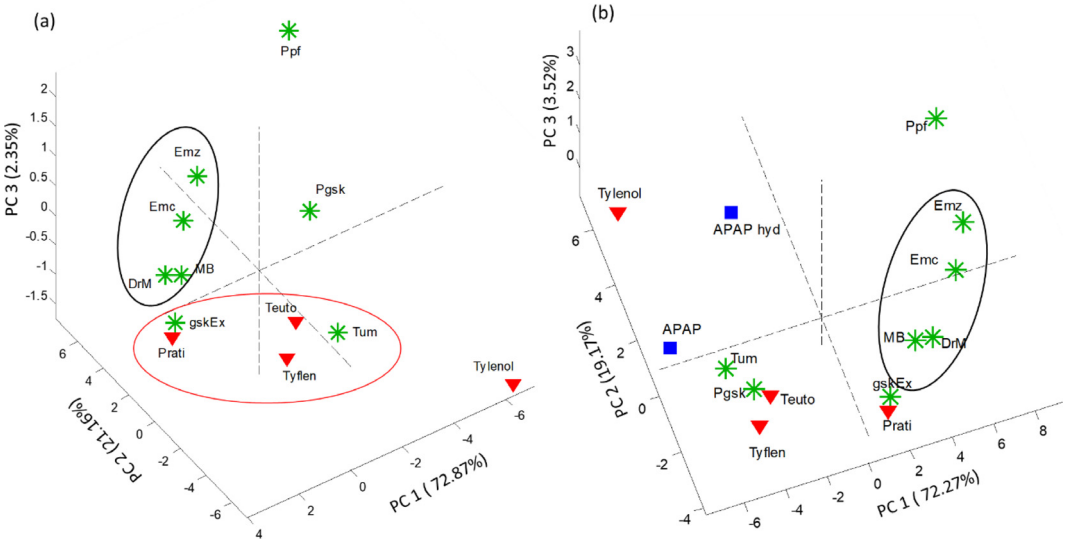

Fig. 6. (a) Comparative plots of powder X-ray diffraction of paracetamol brands (b) PCA-XRD model analysis of paracetamol brands vs. APAP forms.

Herein, the diffraction region between 21 and 32 ($2°\theta$) that presents significant differences in the diffraction peak positions, intensities, and presence of new peaks was selected for comparative PCA analysis.

Fig. 6a presents a 3D PCA-XRD score plot/projections for the 12 paracetamol brands with the PC1 (72.87%), PC2 (21.16%), and PC3 (2.35%) revealing important information such as the crystallinity level of the active ingredient (APAP form I) and relationship (correlation) between the brands. The PC1/PC2 strongly project Tylenol away from brands like Teuto, Prati, and gskEx (red circle), and indicate or suggest differences in crystallinity and composition. Likewise, the PC2/PC3 indicates a relationship between Emz, Emc, MB, and DrM (black circle), while Ppf and Pgsk are well projected away from the brands/groups by the model. However, brands that correlate are expected to present similar properties like solubility and dissolution rate, and this effect can be optimized through formulation methods and the addition of excipients.

The Fig. 6b presents the PCA-XRD model for brands vs. APAP (form I and hydrate), and the PC1 (72.27%)/PC2 (19.17%) indicates a strong relationship (correlation) between Tum, Pgsk, Teuto, and Tyflen based on their APAP crystallinity and excipient content. Likewise, the model projects Tylenol alongside pure APAP samples to indicate its level of crystallinity and shows the projection of Emz, Emc, DrM, MB, and Ppf as observed in both models, to be brands that present low crystallinity. The PC2 (19.17%) and PC3 (3.52%) show non-correlated samples projection based on differences in crystallinity as observed with APAP hydrate, Ppf, and brands in the black circle, through analysis of integrated diffraction peaks intensities, width, and positions. The PCA-XRD models strongly discriminate between the brands, thus indicating that brands with similar diffraction patterns between 5° and 45° (2θ) are grouped, and are expected to present similar physicochemical properties like solubility and dissolution rate for the APAP (API).

APAP presents low bioavailability and the manufacturing processes generally involve optimizing its solubility profile by different formulation strategies that include the addition of excipients/polymers, which often alters the APAP diffraction pattern. As reported in the literature, Leyk and Wesolowski [37], confirmed the amorphization and reduction of paracetamol crystallinity with hydroxypropyl methylcellulose (HPMC), Salunkhe et al. [38], reported the amorphization or/and polymorphic transformation of paracetamol, while the work by Tian et al. [39], indicates the dissolution of paracetamol in hypromellose. In this report, comparative differences in diffraction peak positions and the presence of new peaks are observed for Emc, gskEx and Emz (at 9.55° ± 0.05°), Pgsk (29.60°), Ppf (29.80°), and Prati (21.0°), while varied peak intensities (APAP crystallinity) is observed. The structure-property effects of observed diffraction variation on drug release are investigated through dissolution studies.

Dissolution study

The United States Pharmacopeia (2012) [40] guidelines and specified conditions for dissolution testing and the expected percentage release (minimum dissolution of 80% of the labeled amount dissolve within 30 min in the selected media) as a function of time, thus it reveals drug pharmacokinetic parameters like bioavailability and serves as a quality control measure in pharmaceutical development. The percentage dissolution rate (drug release) for the brands and modeled 400–500 mg/tablets was monitored in dissolution apparatus containing 900 mL of distilled water at 37 ± 0.5 °C, at a stirring rate of 60 rpm, and the average of six consecutive determinations is presented as a plot of percentage cumulative drug release (%CDR) against time in Fig. 7. The%CDR after 5 min presents minimum and maximum of 40.99% and 50.16%, respectively, with brands like Tyflen, Tum, Tylenol, and Pgsk showing higher drug release compared to others. At 20 min, more than 80%

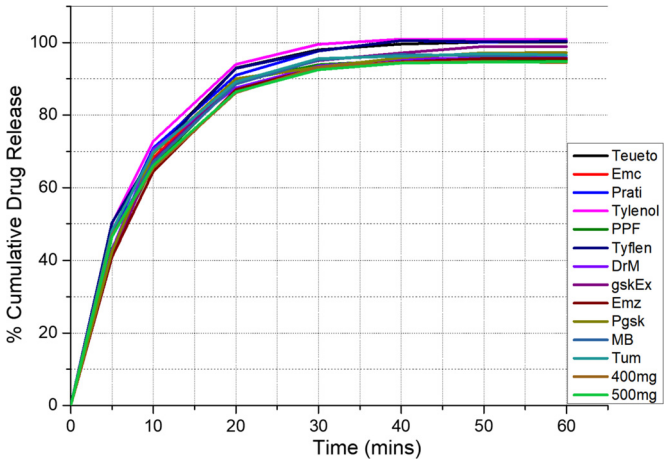

Fig. 7. Comparative dissolution rate studies of paracetamol brands and model tablets.

CDR is achieved, which includes the prepared 400–500 mg/tablets. At 30 min, the maximum and minimum%CDR of 99.5% and 92.5% respectively are observed at a stirring rate of 60 rpm and considerably above the designated pharmacopeia specified standard. Nonetheless, the result of the dissolution studies indicates that brands with observed diffraction variations (enclosed in the black circle) in Fig. 6a/b present a lower drug release profile. More so, the visibility studies on price indicate that some paracetamol brands are 2 to 3 times more expensive than others, with the quality of used API and manufacturing standard being the reasons for the higher price.

Conclusion

In this work, we highlighted an advanced screening methodology through the combination of spectroscopic (Raman/FTIR) and X-ray diffraction techniques with PCA as a predictive screening tool to investigate and verify latent chemical information of all pharmaceutical solid drugs. Herein, the developed FT-IR/Raman–PCA models present no evidence of drug falsification in 12 paracetamol brands but correlate similar brands based on characteristic vibrational and absorption modes of the API (APAP form I). The XRD-PCA model unveiled certain discrepancies concerning the diffraction patterns and expected qualities of APAP form (I) in some brands. However, at a stirring rate of 60 rpm, the brands present dissolution profiles above the recommended drug release limit of 80% CDR in 30 min. The lower%CDR observed in some brands is due to factors such as structural modification and quality of the APAP form (I) as unveiled by XRD-PCA models. Nevertheless, these presented results serve the evidence of improvement in the formulation quality of simple OTC generic drugs marketed at registered drug stores in developing countries. This research presents a new screening technique for monitoring the quality of pharmaceutical solid drugs and was able to conclude that structure-property relationships and solid drug quality can be unveiled and predicted through this advanced screening technique. Finally, it is expected that the advanced screening technique demonstrated/presented herein will be utilized to investigate the structure-property and quality of finished drugs e.g. antimalarial drugs that present increasing global challenges.

Author contributions

This manuscript was written through the contributions of the authors and approved the submission of the final version for publication.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We will like to thank Professor Dr. Renato Lajarim Carneiro of the Federal University of São Carlos – UFSCar, Department of Chemistry, and Professor Dr. Gabriel Lima Barros de Araújo of the University of São Paulo – USP, Department of Pharmaceutical Science, for research equipment assistances and supports.

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