dx.doi.org/10.14227/DT290322P138

Contribution of Multivariate Analysis to the In Vitro Dissolution Profile for Testing Clopidogrel Drugs Similarity

El Khabbaz Choukri[1], El Orche Aimen[2*], Cheikh Amine[3], Bouchafra Houda[4], Moulay El Abbes Faouzi[5], Cherrah Yahia[3,5], Boussen Ratiba[1], and Mustapha Bouatia[6]

[1]Laboratory of Nanotechnology Materials and Environment, Mohammed V University; Faculty of Sciences, Rabat, Morocco.
[2]Team of Analytical & Computational Chemistry, Nanotechnology and Environment, Faculty of Sciences and Techniques, University of Sultan Moulay Slimane, Beni Mellal, Morocco.
[3]Abulcasis University of Health Sciences, Cheikh Zaid Hospital, Rabat, Morocco.
[4]Laboratory of Drug Science, Biomedical Research and Technology, Faculty of Medicine and Pharmacy, Hassan I University, Casablanca, Morocco.
[5]Laboratory of Pharmacology and Toxicology, Bio Pharmaceutical and Toxicological Analysis Research Team, Faculty of Medicine and Pharmacy, Mohammed V University, Rabat, Morocco.
[6]Laboratory of Analytical Chemistry & Bromatology, Faculty of Medicine and Pharmacy, Mohammed V University in Rabat, Rabat, Morocco.

e-mail: aimen.elorche@gmail.com

ABSTRACT

A novel approach to test the similarity of clopidogrel batches by comparing drug dissolution profiles, based on the combination of principal component analysis with hierarchical cluster analysis (PCA-HCA), is presented. Dissolution curves corresponding to five brands of clopidogrel drugs, taken as model drugs, were prepared by measuring the dissolution rate in pH 1.2, 4.5, and 6.8). The dissolution data were analyzed by similarity factor (f_2) calculation and the PCA-HCA method, and the results were compared. Unlike the f_2 test, the PCA-HCA approach reflects the variability inside the individual dissolution patterns, which it is also sensitive to profile variations (form and size). The comparison between the PCA-HCA results with those of f_2 tests gives approximately similar results, knowing that PCA-HCA represents, in general, a more discriminative criterion.

KEYWORDS: Clopidogrel, dissolution, similarity, multivariate analysis

INTRODUCTION

Clopidogrel belongs to the second class of the biopharmaceutical classification system (BCS) with low solubility and high permeability; its solubility is very sensitive to the pH value (*1*). It is an inactive prodrug that is absorbed from the intestine and subsequently metabolized in active moiety (*2*). It is extensively used for reducing the risk of atherosclerotic events associated with platelet aggregation, stroke, and vascular-related death (*3*). Clopidogrel is dedicated for patients with acute coronary syndrome and those with atherosclerosis who have suffered from a myocardial infarction, stroke, or have peripheral artery disease (*4*).

Generally, clopidogrel requires metabolic activation in the liver. Up to 85% of the absorbed drug can be converted by carboxylesterases to a predominant metabolite carboxylic acid derivative that is considered inactive (*5*). The active metabolite clopidogrel is available in low quantity, whereas the remaining types of clopidogrel are hydrolyzed to an inactive acid derivate compound by esterase paraoxonase-1 (*6*).

The efficacy of clopidogrel can be affected by inter-individual variability in drug treatment. This variability is attributed to the clopidogrel P2Y12 receptor polymorphism; the hepatic metabolism variable is essential for its biotransformation and low oral bioavailability (*7*). This later can be related to its low solubility and further impact on intestinal absorption. These factors may be the main reasons behind the clinical limited effectiveness of this drug (*8*). As clopidogrel faces protonation in the stomach, only the non-ionized form can be absorbed in the intestine where factors such as solubility, limitation, and precipitation in the intestinal pH can limit the protonation process (*9*). Furthermore, efforts

*Corresponding author

to improve dissolution of clopidogrel in the intestines, the primary site of drug absorption, are needed and remain a challenge for clopidogrel management (*10*).

Clopidogrel was genericized after its pharmaceutical patent expired in May 2012. Several generic drugs are now available on the international market. It is critically important to demonstrate that these preparations are bioequivalent to the original drug in view of the above-mentioned elements. For this reason, the pharmaceutical industries try to respect as much as possible the similarity in excipients composition compared to those used in the reference product and attempt to have a similar manufacturing process to minimize the sources of variability between the generic and the originator drug (*5*).

However, more importantly, the commercially available salts of clopidogrel (bisulfate, besylate, hydrogen sulfate, etc) differ on their physicochemical properties. For instance, the bisulfate clopidogrel form of salt has been reported to have poor stability and degrades under moisture and heat conditions (*6*).

Clopidogrel base is a white to off-white powder with chemical formula $C_{16}H_{16}ClNO_2S$ ((αS)-a-(2-Chlorophenyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-acetic acid methyl ester), and it has a molecular weight of 321.826 g/mol. It is soluble in methanol, sparingly soluble in methylene chloride, and practically insoluble in ethyl ether (*4*). The pKa value of clopidogrel is about 4.56 ± 0.20 (*11*). Similar to all bases, clopidogrel is practically insoluble in water at neutral pH, and it is freely soluble at pH 1. This feature is one of the reasons why the hydrogen sulfate salt is the preferred form of the active ingredient (*12*). The interaction site for salt formation is at the pyridine nitrogen, which is only capable of forming salts with extremely strong acids. Clopidogrel bisulfate has six different polymorphs and one amorphous form, but only I and II forms are used in pharmaceutical formulations (*13*). Polymorphic I (first) form has a melting point range between [198 and 200] °C, while the II (second) form has a melting point between [176 and 178] °C (*11*).

In pharmaceutical development, comparative study of the dissolution kinetics of an originator and a generic drug has an important place in early development. Later on, the dissolution test is a key parameter of quality control and is used to assess reproducibility between batches of drug products. Combined with other pharmaco-technical tests, dissolution studies ensure the quality, efficiency, and safety of drug products use.

The in vitro dissolution study, as a routine quality control test, must be robust, reproducible, and discriminatory to ensure consistent product quality and to detect alterations in product quality that may affect the in vivo drug performance (*14*).

The objective of this work is to evaluate the dissolution profile of five generic brands of clopidogrel available on the Moroccan market with the originator brand in three different pH dissolution media (pH 1.2, 4.5, and 6.8), with pH 4.5 being close to pKa of the base. Subsequently, the dissolution data will be analyzed to determine and compare similarity using the similarity factor (f_2) calculation and the PCA-HCA approach.

MATERIALS AND METHODS

API Reference and Various Drug Products

The Standard of clopidogrel bisulfate was provided by Medispray, India.

The reference product, Plavix (R), and five generic products (T1–T5) of clopidogrel (75-mg tablets) were purchased from the Moroccan market. All of them are formulated with the bisulfate salt form of clopidogrel. Information on the generic drugs studied is provided in Table 1.

Table 1. General Information about Generic Bisulfate Clopidogrel (75 mg) Products Used in This Study

Generic Name	Batch No.	Expiry Date	Code
Pedovex	ET11/17	05/2022	C01
Agreter	CRR1S0290318	02/2023	C02
Pedovex	AAIH001125	02/2020	C03
Ceruvin	AALH009032	04/2023	C04
Agrel	7010818070	12/2020	C05

Preparation of Buffer Solutions

Three buffer solutions were prepared as dissolution media according to *United States Pharmacopeia* (USP) requirements (15). The first buffer solution was prepared at pH 1.2, which consisted mainly of a mixture of potassium chloride solution (0.2 M) and hydrochloric acid (0.2 M). The second buffer solution was prepared at pH 4.5, which consisted mainly of a mixture of sodium acetate tri-hydrate and acetic acid (2 M). The third buffer solution was prepared at pH 6.8, which consisted of a mixture of monobasic phosphate monobasic phosphate (0.2 M) and a solution of sodium hydroxide (0.2M).

Preparation of Standard Solution

A standard solution of clopidogrel bisulfate was prepared according to USP requirements (*15*). A sample (20.83

mg) of clopidogrel bisulfate was dissolved in 25 mL of methanol, the solution was diluted with the previously prepared media, obtaining a solution with a concentration of 0.0830 mg/mL, and the solution was filtered before characterization within the spectrophotometer.

Dissolution Test

The dissolution test was performed according to the USP guideline (*15*).

In vitro dissolution tests were performed using a SOTAX AT7 Smart semi-automated dissolution tester with the paddle setting (USP apparatus 2), 50 rpm ± 4%, 900 mL of dissolution media, 37 ± 0.5 °C. Six tablets of the finished product were weighed. After the stabilization of the conditions of the apparatus, the tablets were placed in the vessel at the same time to carry out the dissolution test according to the protocol. Samples (xx mL) were collected at 5, 10, 15, 20, 30, 45, and 60 minutes.

The amount dissolved was determined by UV absorption spectroscopy at a wavelength of 240 nm in a filtered portion of the solution under test in comparison with the standard solution. All samples were analyzed with a JENWAY 6705 UV/VIS spectrophotometer.

Comparison of Profiles

The similarity factor (f_2) analysis is the simplest and most widely applicable among the studied methods for comparing dissolution profiles. Moore and Flanner proposed a model-independent mathematical approach to compare the dissolution profile using the difference and similarity factors, f_1 and f_2, respectively, but f_1 is neither described nor requested in the majority of the international guidelines (*16*).

The f_2 is inversely proportional to the average of the difference squared between two dissolution profiles, emphasizing the larger difference among all time points. The f_2 measures the proximity between the two profiles without taking into account the shape. f_2 has been widely accepted since the regulatory interest is in knowing whether the dissolution profiles of the test and reference products are similar or not.

When the two profiles are identical, $f_2 = 100$. The agencies have established a standard of f_2 between 50 and 100 to indicate acceptable similarity between two dissolution profiles. The value of 50 corresponds to a mean difference of 10% between the curves.

For pharmaceuticals dissolving to 85% or greater within 15 minutes, the profile comparison is not necessary.

For a dissolution profiles comparison, at least 12 units should be used for each profile determination, the average of which are used to estimate f_2. The percentage coefficient of variation at the early point (first or before 10 minutes) should not be greater than 20%, and at the other time points it should not be greater than 10%. Because f_2 values are sensitive to the number of dissolution time points, only one measurement should be considered after 85% dissolution, per EMA and US-FDA reference tests.

For the scope of this work, the f_2 was calculated using only 6 tablets for each formulation. The value obtained will give an analysis trend of the similarity between the profiles and will allow for comparison between the adapted approach and other methods.

Multivariate Data Analysis

Th Principal component (PC) analysis (PCA) is one of the most widely used methods of exploratory multivariate data analysis (*17, 18*). It is used to explore multidimensional data sets composed of quantitative variables. PCA can be considered as a projection method that allows to project the observations from the p-dimensional space of the p variables to a k-dimensional space ($k < p$) such a quantity of information is preserved (the information is here measured through the total variance of the scatterplot) on the first dimensions. If the information associated with the first two or three axes represents a sufficient percentage of the total variability of the scatterplot, then the observations can be represented on a two- or three-dimensional graph, which greatly facilitates the interpretation (*18*). The main objective of PCA is to study the similarity between individuals and the link between variables. PCA is performed in the dissolution data tables (the variables are the sampling times (column), and the individuals are the tablets of each drug (row)).

The number of significant PCs to retain can be obtained by various means, including cross-validation, by setting a threshold at the minimum explained variance, or by evaluating the residual variance (*19*). Observing the shape of the PCs is also a useful index. In this work, the total variability explained by the PCs was used with an increasing number of PCs until the optimal number of factors resulted in a low residual variance. In our study the first three PCs were selected arbitrary to be used as variables for the hierarchical ascending classification (HCA) analysis.

The HCA is an iterative classification method of simple principle (*20*). The HCA principle is to gather individuals according to a criterion of similarity defined beforehand, which will be expressed in the form of a 2 × 2 similarity matrix, expressing the similarity between two individual data points at a time. The main function of HCA is to group samples so that those belonging to the same cluster are more similar than samples from other groups.

The HCA is usually displayed as a dendrogram (*21*). This dendrogram represents a hierarchy of partitions. We can then choose a partition by truncating the tree at a given level of similarity, the level depending either on the user's constraints (the user knows how many classes he/she wants to obtain), or on more objective criteria.

In general, there are several calculation methods used for clustering analysis, among them we find the McQuitty's linkage method. This method has been considered as the best clustering algorithm (*22*). Based on McQuitty's linking method, the distance is calculated with the following distance matrix:

$$dmj = \frac{dkj - dij}{2}$$

Where *dmj* is the distance (*d*) between clusters *m* and *j*, *m* is the merged cluster that consists of clusters *k* and *i*, so *m* = (*k*, *i*); *dkj* is the distance between clusters *k* and *j*; and *dij* is the distance between clusters *i* and *j*.

A flow chart of the main procedures applied to develop this study is presented in Figure 1.

The PCA analysis was performed using Unscrambler software 10.4, and the HCA analysis was performed using Minitab 17 statistical software.

RESULTS AND DISCUSSION

The dissolution results are presented in Figure 2. The raw dissolution data are given in Tables 2–4.

The dissolution results at pH 1.2 showed that the dissolved quantity (*Q*) exceeded 85% within 15 min for the reference product (R) and for the generics T1–T3; however, *Q* did not exceed 85% for generics T4 and T5. The f_2 values for T4 and T5 versus R was calculated for the time points 5, 10, and 15 minutes. At pH 4.5, $_Q$ of the five generics did not exceed 85% after 15 min. The absence of complete dissolution could be attributed to the lower solubility of the drug in pH 4.5 compared to pH 1.2. The f_2 values for this pH reveal that only two generics are similar to R (f_2 between 50 and 100) whereas three generics are not ($f_2 < 50$). At pH 6.8, Q decreased for solubility reasons. The calculation of f_2 shows that three generics are similar to R and two were not.

In summary, the comparative study using f_2 analysis showed that only one generic was similar to the originator in all three pH values; two generics were similar at pH 1.2 and 6.8; one generic was similar at pH 1.2 and 4.5; and one generic was not similar to the originator at any pH value. These results do not exclude the in-vivo performance of the drug, but only indicate an in-vitro difference with respect to the behavior between the formulations.

The PCA and PCA-HCA were used to evaluate the similarity between the test and reference drugs. The purpose of these exploratory methods is to investigate the similarity between the samples and the relations between the batches. In both methods, the times within each formulation are closely linked together (i.e., dissolution at

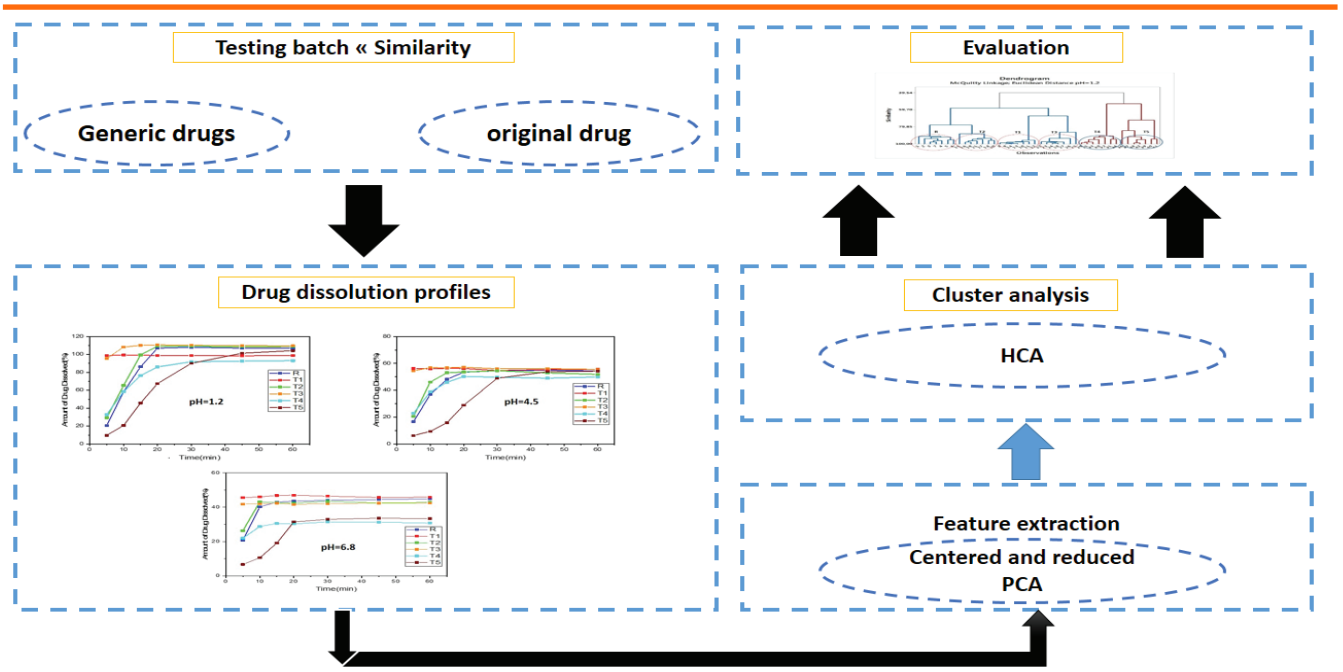

Figure 1. Principal steps employed to study the similarity of the drugs. HCA: hierarchical ascending classification; PCA: principal component analysis.

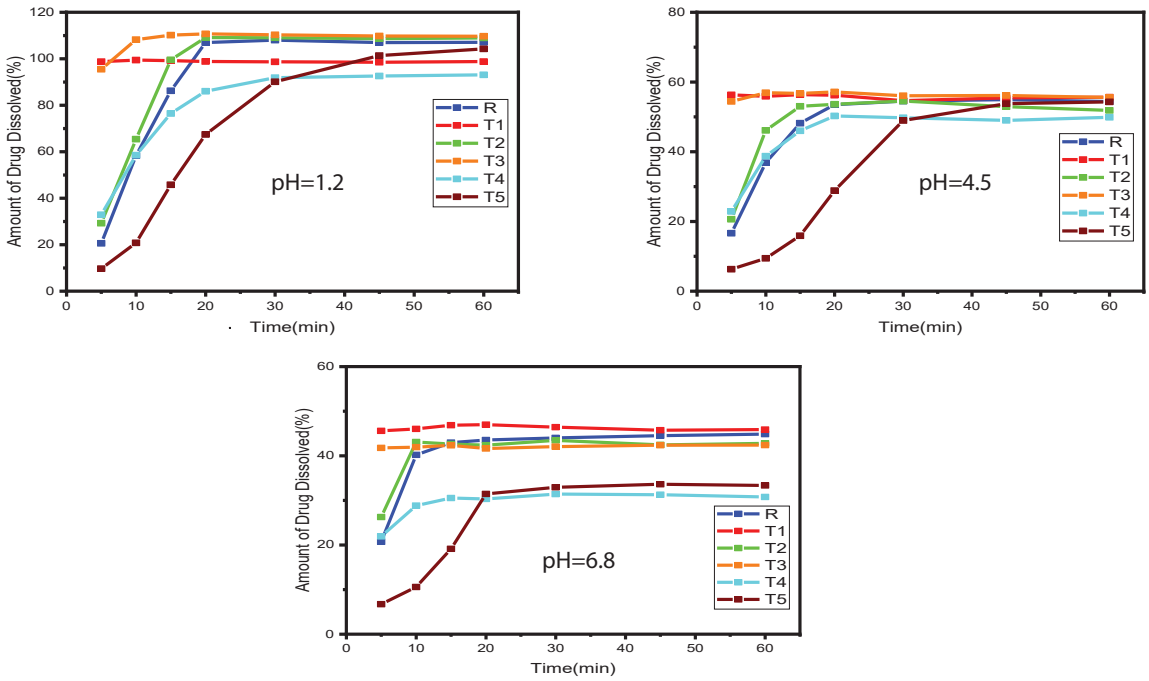

Figure 2. In vitro dissolution profiles of clopidogrel in pH 1.2, 4.5, and 6.8. R: reference; T: test.

time 2 depends on dissolution at time 1 etc...), with the exception of generic T5 with a larger dispersion of various times.

Application of the PCA on the obtained data by the dissolution at pH 1.2 (Fig. 3) shows that the first two PCs present 92% of the total data variability. The score plot PC1-PC2 shows that samples of batch R and T2 are very close to each other, which means that they present the same response pattern regarding the product amount released at different times. This plot also shows that T4 and R are not linked; T1, T3, T5, and R do not have the same response pattern; T1 and T3 are linked; and T1 and T5 are not linked.

PCA analysis at pH 4.6 shows that the first two PCs correspond to 89% of the total data variability. The score plot shows that the samples of batch R and T2 contribute in the same way along the PC1-PC2 axis, which means that they present a similar response behavior, while batch T1 and T2 have a similar response behavior along the PC1-PC2 axis. The T4 and T5 batches do not present the same response behavior compared to R, as they are far from each other.

The results found by the PCA at pH 6.8 reveal that the projection of the weights of the six batches on the first two PCs, which represent 96% of the total variability of the dissolution data, allow us to conclude that T1, T2, and T3 are similar to R because they contribute identically with the PC1 axis whereas T4 and T5 do not present the same pattern of response because they are very distant from R. These results are considered consistent with those found by the statistical approach based on the calculation of the similarity factor which shows that batches T4 and T5 do not have the same dissolution profile as R.

The observation of the results of PCA-HCA in form of dendrogram (Fig. 4) at pH 1.2 obtained on the data generated by PCA (PC1, PC2, and PC3) demonstrates the existence of two main clusters, the left one being sub-clustered in two. Cutting this tree at a certain height produces the desired partition, which is fixed at 50% of similarity. It shows that the four batches T1, T2, T3, and R belong to the first class, and batches T4 and T5 belong to the second class. In term of dissolution rate, T4 and T5 exhibit the slowest dissolution. In the first class, two subgroups exist: R and T2 in first subgroup and T1 and T3 in the second subgroup, this corresponds to faster dissolution. Congruent with the f_2 calculation, the approach developed by PCA-HCA shows that T4 is also not similar to R. The difference is due to the fact that only three points are used and the main difference between R and T4 is located after 15 minutes.

For the results found by the PCA-HCA approach on dissolution results at pH 4.5, there are two main clusters if we set the partitioning index at 50%, which allows us to conclude that all batches are similar to the reference except for the batch T5. However, starting from a

Table 2. Dissolution Results of Clopidogrel in pH 1.2

Sample	Time (min)						
	5	10	15	20	30	45	60
R	26.176691	55.6357511	80.7889512	95.312461	106.41956	106.185813	105.886698
R	23.359757	58.5757602	77.7934234	90.3707965	100.324724	104.328553	104.03467
R	26.2512013	57.9519204	78.2496579	93.2663544	102.51699	105.851828	105.093868
R	18.1797553	53.4659225	78.1528227	92.5087918	102.034715	105.724333	105.32183
R	27.143719	58.2657052	81.174836	95.9384778	104.768767	104.539283	103.792411
R	31.0628171	65.9786475	86.2069197	94.8544856	102.505016	104.038771	103.678424
T1	99.3608238	99.7068229	100.173411	99.4606212	99.3053808	99.0879402	98.870153
T1	98.5587479	99.9897163	99.2535027	98.9110565	98.6339947	99.1349706	98.5971019
T1	100.220627	99.488543	99.3406819	98.8053847	98.4643472	98.5723093	98.3585515
T1	95.6385171	97.918727	97.7232804	97.7232804	98.5050667	97.9838759	99.1565552
T1	99.5335082	100.295687	100.210518	99.866046	99.7150569	99.3065724	99.9870182
T1	99.3535523	99.6712441	99.1304882	99.1815688	98.6420992	98.4954722	99.5189211
T2	18.6649956	51.522327	91.889027	108.52311	108.570029	107.915138	108.448592
T2	33.9326252	66.7640827	92.9713254	108.140296	109.020878	108.506374	108.408401
T2	39.2121018	70.155704	103.650175	109.436483	109.343779	108.752314	109.721804
T2	25.1170693	67.8015686	108.598658	110.849031	110.340883	110.413475	110.340883
T2	34.4555317	67.2998164	91.1821825	108.717643	108.694705	108.670184	109.344108
T2	23.5682527	68.616491	109.71277	109.840462	109.532786	109.081203	109.347937
T3	92.2637903	102.292329	104.710033	106.628905	106.743693	106.306415	106.212533
T3	89.1030825	106.572384	107.832428	108.206243	107.162699	106.86168	106.292921
T3	93.9338695	104.414292	107.253408	107.578061	106.723393	106.354636	107.224265
T3	94.8671575	107.838646	109.731782	109.240969	109.100737	109.030621	109.170853
T3	88.5699995	103.113422	105.021287	105.754005	106.071695	104.821437	104.458335
T3	96.5457046	105.349692	106.52899	107.070644	106.770726	106.540352	106.170891
T4	31.0049567	59.1824205	77.4348137	86.2547246	91.6888101	92.5982165	92.8610288
T4	36.7072952	62.7435471	80.7909712	89.9155772	92.4198898	92.2187607	92.2505526
T4	27.8921975	53.5756089	73.9074582	81.5204393	90.8353283	92.3790104	92.6395599
T4	33.4129366	61.5241895	79.7101974	89.8820324	93.7658239	93.8891189	94.9987736
T4	37.2040637	59.3965408	75.4064135	86.9267922	91.3040694	92.3307794	93.0594493
T4	31.0761955	54.9504844	71.9863758	82.5439735	92.374553	93.4448191	94.447321
T5	8.95298226	20.8322634	39.8997207	58.5972689	77.6485151	100.319456	103.232307
T5	9.34335762	18.5687268	40.0652599	56.9072326	89.7742785	100.630686	103.996209
T5	9.77177873	21.8595864	44.6479319	68.8145605	90.4293022	101.113517	105.633148
T5	10.6067178	19.3147022	48.3194924	67.6996682	85.6394255	102.728026	106.590967
T5	8.97586903	22.7722344	57.4939611	76.5753826	99.3996829	103.415908	103.968283
T5	10.4278478	21.521736	44.6300154	76.5099336	98.7470258	101.463436	104.033182

R: Reference; T: Test

partitioning index equal to 56% we obtain three clusters, the first cluster contains R, T2, and T4, a second cluster contains T1 and T3, and a third contains T5. These results show that the batches T2 and T4 are closer to R than the others. Going ahead, we find that the formulation T2 is closer to R than T4. This finding agrees with the statistical calculation that showed batches T2 and T4 have a f_2 of 67.27 and 65.62, respectively. Dissolution at pH 4.5 showed many differences, which is probably because this pH is close to the pKa of clopidogrel, increasing the possible influence of the composition of the formulation on the dissolution and slight pH changes.

For the results obtained by PCA-HCA at pH 6.8, we obtained similar results as for pH 1.2: two classes in case of a partition index at 50%. Clusters are linked with

Table 3. Dissolution Results of Clopidogrel in pH 4.5

Sample	Time (min)						
	5	10	15	20	30	45	60
R	13.0540835	29.7437513	42.7998127	51.5458289	53.5138309	54.3777299	52.5908149
R	13.6611738	38.6519439	48.5752688	53.3229238	54.7444184	54.5123196	52.7967303
R	18.7497174	37.6370495	50.7126876	54.4981011	54.105686	54.8303518	55.0732541
R	17.2708514	38.2197545	48.6142484	53.4916648	53.9714106	55.6505212	55.1707753
R	20.0250862	41.0396009	49.3141837	54.336442	54.9638685	55.1204513	55.0427074
R	16.891162	35.8236698	49.0513352	53.7113839	55.7566948	55.1308759	55.5214345
T1	56.1950191	54.4913353	55.8015995	56.131718	54.1975185	55.1728996	55.2584354
T1	56.6481775	56.4085932	57.2354569	57.7297427	54.9587187	56.2679577	55.2986261
T1	57.8472904	56.9512219	56.385176	56.3901833	55.5840175	55.5086813	55.8357451
T1	57.4306511	56.2721077	57.7616635	57.0996387	55.692836	57.347898	57.8444166
T1	55.4649328	56.770681	56.127295	56.535059	54.6878092	54.1332051	55.5801458
T1	54.1908626	54.429672	54.8216144	53.3521015	52.7393526	54.2082148	53.9787322
T2	21.2930303	46.9671389	53.7633622	54.0056683	55.4199266	53.7811724	52.3064476
T2	20.9845011	40.8448634	52.1698015	53.3343117	53.9534841	52.1924699	50.9754984
T2	22.8249256	52.2815871	53.2205078	52.0675764	54.1557498	52.697707	51.4002264
T2	15.3720222	39.4292369	51.8805749	53.0334765	53.5714973	52.3417355	52.1111552
T2	18.5054352	44.6167083	52.9560833	53.8921998	54.5133855	53.1282975	51.9037114
T2	24.7273058	52.5472805	54.129275	55.2315581	55.6237102	53.4419665	52.28153
T3	53.8603636	56.3366105	56.3383836	57.4470649	55.6291114	55.4728498	56.0227575
T3	55.477883	57.4114675	56.6109702	57.570717	56.3743325	56.2953788	55.4242033
T3	53.9855224	56.5822749	56.8159586	56.7352951	55.2534039	55.6414194	54.6334535
T3	54.4573453	56.8423385	56.9218383	57.0808379	56.3653399	56.2063403	56.1268406
T3	54.4765349	57.3957666	57.5499414	57.7023616	57.4626561	57.534809	56.2082045
T3	54.2968565	56.930685	56.1550156	56.2289197	55.1509547	55.6079532	55.0699403
T4	23.5365516	40.4225745	42.9918906	51.138198	50.4292411	48.8369825	50.5477411
T4	23.4706072	36.0394738	44.5806182	49.5298844	49.1502128	49.0922356	49.1136668
T4	25.3030005	43.8252408	50.1593143	52.2477829	50.9491082	48.6719769	50.1717993
T4	21.7225417	39.0352441	46.46664	50.8764793	49.4065328	50.3048334	50.1415061
T4	20.4239202	37.2999073	45.7184336	49.9591781	48.7895861	48.0206856	50.3269422
T4	22.6994803	35.6743678	46.0241544	47.7250558	49.653665	48.9605153	48.9803451
T5	6.32333934	9.61630612	20.5938867	31.7451395	44.7876856	52.3003836	53.3966486
T5	5.83974903	8.02702439	11.0652722	21.9904693	37.1464156	53.3063427	53.3889848
T5	6.47107325	10.5453066	13.7334939	32.242254	52.051805	53.6176206	54.320188
T5	5.95671141	8.73651007	13.6607248	26.2095302	53.5309799	54.5634765	55.3577047
T5	6.95567327	9.3010174	17.213974	33.1560346	54.8707771	54.7166457	53.8629949
T5	6.17266009	10.3381065	19.0446787	27.70245	51.2585783	54.2360954	55.406219

R: Reference; T: Test

dissolution rate, the first cluster contains R, T1, T2, and T3, whereas the other cluster contains T4 and T5, which demonstrates that the batches T1, T2, and T3 have a similar relationship with R while the batches T4 and T5 do not. Again, the first cluster could be divided in two subgroups, R and T2 in one subgroup and T1 and T3 in the second. These results are exactly the same as those obtained by the statistical analysis, which shows that T1, T2, and T3 have a value of $f_2 > 50\%$ compared to the T4 and T5, which have an f_2 less than 50%.

These results have reported a certain similarity and complementarily between the in vitro dissolution method and other statistical methods for assessing similarity. The latter could be used to support the dissolution results especially in cases where the factor is very close to 50

Table 4. Dissolution Results of Clopidogrel in pH 6.8

Sample	Time (min)						
	5	10	15	20	30	45	60
R	17.6307533	40.2915767	43.3936915	44.4380687	44.4588828	44.7686453	43.7756911
R	17.2743217	36.9135803	42.0075697	42.6099936	43.495484	43.2301595	45.3922723
R	19.2273943	40.8945145	44.0669511	44.3907458	45.0091269	45.3270799	46.2333711
R	21.0715282	40.2668245	42.287382	42.8646842	44.5965906	44.8852417	46.328497
R	24.5485943	41.3835398	43.593238	44.3408985	43.4941993	45.1011419	44.1119731
R	24.5038041	41.4839273	42.3600807	42.52431	42.9687185	43.831417	43.2876795
T1	44.7082201	44.7292555	48.804451	46.6463045	48.0997666	45.5233402	47.686341
T1	46.0170824	49.0740006	45.9057898	46.4973343	47.3719492	45.9505487	45.9638555
T1	46.6645255	46.1026891	49.1350004	50.0006298	47.00032326	45.8739415	46.5971052
T1	47.1031821	45.7987863	46.5234506	46.8133164	45.7987863	46.9582492	45.9437192
T1	45.7267538	46.7578235	47.0606524	46.7852435	47.9461546	46.236845	45.6783657
T1	43.263989	43.6899355	43.7043872	45.0757826	42.2417175	43.8781886	43.3506995
T2	23.6526395	42.8591916	42.5963845	41.4762494	42.9288996	42.2394316	42.9713654
T2	25.7214079	43.8899308	42.9150577	41.8032419	42.1100729	41.5690501	42.0140146
T2	24.8735614	42.0535524	42.6471982	41.961031	41.2780405	41.5849891	41.8895553
T2	33.8525341	44.6970688	42.3523046	43.6712345	48.2142152	43.96433	44.2574255
T2	24.0243902	42.6563139	42.9745231	43.4355603	43.7484796	43.0477682	43.2146043
T2	25.4389748	42.4238422	42.3056699	41.902447	42.4957037	42.2346068	42.3982914
T3	41.9852646	42.2458338	42.3789202	42.1355122	42.0174855	42.2724511	41.6574517
T3	42.1978174	41.8286216	42.7172183	41.720881	42.1037637	42.982183	41.7429906
T3	41.8320376	41.7158375	41.7234458	41.1130495	40.9978869	41.8648918	42.3590881
T3	41.1328949	41.3777336	41.5001529	41.1328949	41.5001529	41.7449916	42.1122496
T3	41.3304055	41.8458067	43.11184	41.9887007	42.7460035	42.7505676	43.3758603
T3	42.0887615	42.6020562	42.6090781	41.7380219	42.9959801	42.6259304	43.127288
T4	21.6528354	28.9608099	33.5744373	30.7330015	32.8794714	33.0306977	31.3829286
T4	20.0617305	31.5517568	29.776532	30.0892688	31.0906918	30.7080884	30.4252945
T4	26.0692665	28.9189786	30.4461414	32.2649275	31.6749447	31.3866896	30.6027591
T4	18.8858169	27.4969666	29.3561921	29.845462	31.6068335	31.5089795	30.7261478
T4	24.606346	27.8751623	31.2232658	29.6719724	31.3408294	30.1851514	31.3583273
T4	20.2733913	28.205287	28.815137	29.3230706	29.8277077	30.7191326	30.0491217
T5	6.5582978	9.44678267	16.4599395	28.8018579	32.8389123	33.5679013	34.0875336
T5	6.59879493	9.04813929	11.8938696	31.4920614	32.3214933	32.1290284	33.1537892
T5	6.49856556	9.92981981	24.1821174	31.7023437	33.1683059	35.5557718	33.1881044
T5	6.92188011	10.9510342	15.0834999	32.6464793	32.9564142	32.9564142	33.2663492
T5	7.25574645	13.4225467	31.4760357	32.6394672	32.8600021	34.945299	33.81283
T5	6.55303432	10.6839308	15.7226594	31.3574828	33.4297209	32.5152433	32.5259338

R: Reference; T: Test

and where bioequivalence is not required. The main issue would be to understand the reason of those dissimilarities and the possible impact in vivo. Furthermore, the selection of the most appropriate media to reflect in vivo behavior is mandatory. A similarity in all pH could be seen as a promising indication of absence of differences in vivo whereas a difference in one only one condition could be inconclusive. For instance, one formulation that was not equivalent in one pH successfully passed bioequivalence. The reason for this difference could be linked with formulation composition and/or interaction between some excipient and dissolution media or excipients and API. For example, it is well documented that sodium croscarmellose interact with basic components as a function of pH value and its ionization (*23*, *24*).

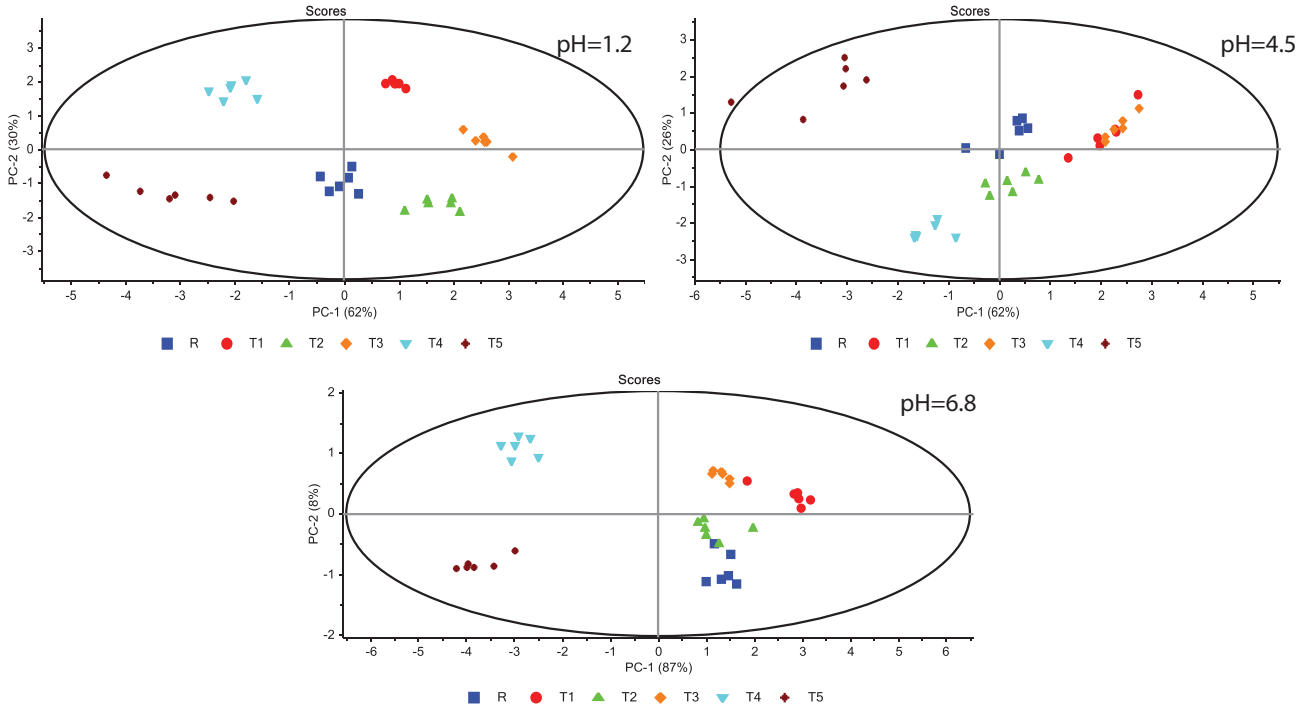

Figure 3. Score plot of PC1 versus PC2. Top left (pH 1.2): PC-2: 30%, PC-1: 62%. Top right (pH 4.5): PC-2: 25%, PC-1: 62%. Bottom (pH 6.8): PC-2: 8%, PC-1: 87%. PC: principal component.

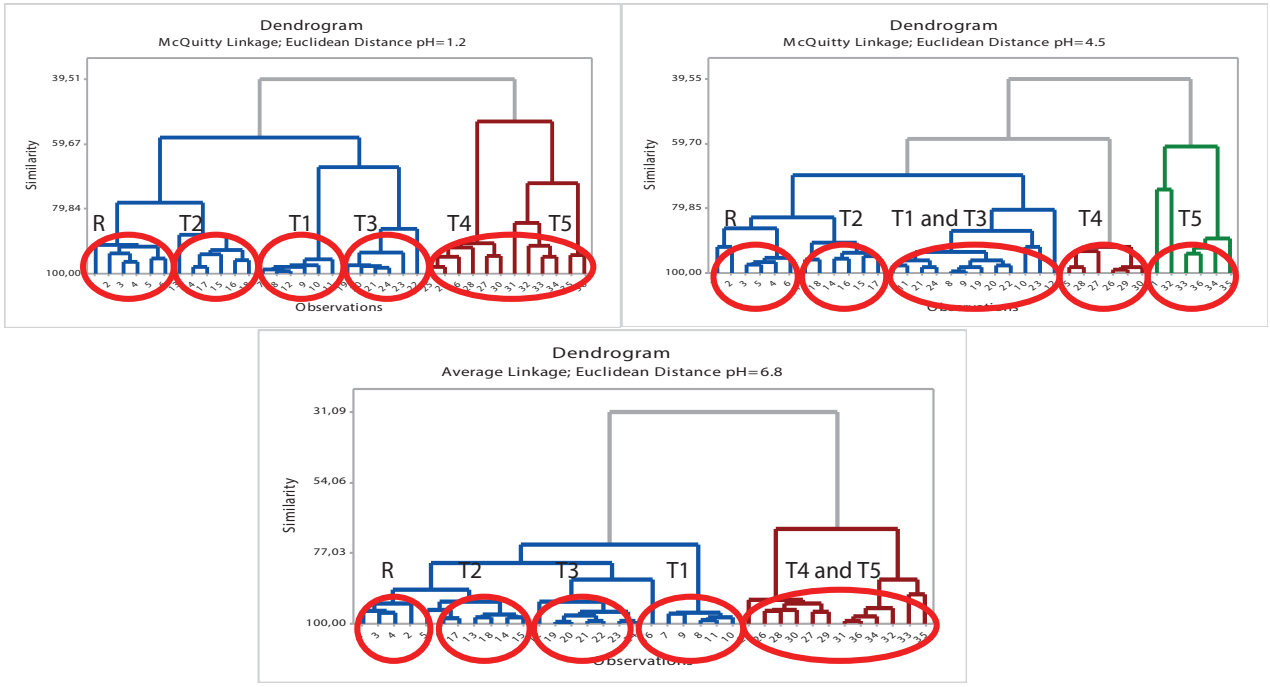

Figure 4. Hierarchical clustering analysis (HCA) generated by the three principal components of the principal component analysis (PCA); R: reference; T: test.

Furthermore, these results reinforce the utility of bioequivalence as a tool for assessing the quality of generic drugs in vivo. Inconclusive results on in vitro dissolution tests could not always preclude absence of bioequivalence. However, nonequivalent in vivo dissolution behavior could have considerable clinical consequences and should prompt the authorities to carry out the necessary investigations to guarantee the quality of the products placed on the market.

Overall, the current dissolution study was able to discriminate between formulations. One formulation was similar to the reference in all pH levels, and all other formulations showed a difference in at least one pH compared to the reference.

The PCA-HCA method allowed for cluster-based analysis of formulations to estimate the overall similarity of the formulation not only based on the distance between formulations but also on the global dissolution curve including the shape.

In contrast to the f_2 calculation, the PCA-HCA approach provides a simple graphical and analytical method for assessing drug similarity by employing robust mathematical and statistical procedures. Moreover, this approach can use all data sets obtained by the dissolution test, regardless of the dissolved drug quantity and data variability. This is extremely advantageous, as it allows a better appreciation of the dissolution behavior of the compared batches.

CONCLUSION

The dissolution test was used in this work to compare the in vitro dissolution profile and more precisely the amount released of the active ingredient between the originator and five different generic products of clopidogrel in three dissolution media (pH 1.2, 4.5, 6.8). The f_2 calculation gives an idea of the similarity between the generic drugs and their originator. This technique could be complemented by other analyses such as PCA and HCA to provide additional evidence of similarity.

CONFLICT OF INTEREST

The authors disclosed no conflicts of interest related to this article.

FUNDING

The authors disclosed no financial support for this article.

REFERENCES

1. Qureshia, M. J.; Phina, F. F.; Patrob, S. Enhanced solubility and dissolution rate of clopidogrel by nanosuspension: formulation via high pressure homogenization technique and optimization using Box Behnken design response surface methodology. *J. Appl. Pharm. Sci*. **2017**, *7* (2), 106–113. DOI: 10.7324/JAPS.2017.70213.
2. Sangkuhl, K.; Klein, T. E.; Altman, R. B. Clopidogrel pathway. *Pharmacogenet. Genomics* **2010**, *20* (7), 463–465. DOI: 10.1097/FPC.0b013e3283385420.
3. Bhatt, D. L.; Fox, K. A.; Hacke, W.; Berger, P. B.; Black, H. R.; Boden, W. E.; Cacoub, P.; Cohen, E. A.; Creager, M. A.; Easton, J. D.; et al; CHARISMA Investigators. Clopidogrel and aspirin versus aspirin alone for the prevention of atherothrombotic events. *N. Engl. J. Med*. **2006**, *354* (16), 1706–1717. DOI: 10.1056/NEJMoa060989.
4. Oldenburg, P. J. Clopidogrel. In *Reference Module in Biomedical Sciences*; Elsevier, 2018; p B9780128012383982000. DOI: 10.1016/B978-0-12-801238-3.98129-7.
5. Zaid, A. N.; Al Ramahi, R.; Bustami, R.; Mousa, A.; Khasawneh, S. Comparative fasting bioavailability of two clopidogrel formulations in healthy Mediterranean volunteers: an in vitro-in vivo correlation. *Drug Des. Devel. Ther*. **2015**, *9*, 2359. DOI: 10.2147/DDDT.S78658.
6. Abd-Elhakeem, E.; Teaima, M. H. M.; Abdelbary, G. A.; El Mahrouk, G. M. Bioavailability enhanced clopidogrel-loaded solid SNEDDS: development and in-vitro/in-vivo characterization. *J. Drug Deliv. Sci. Technol*. **2019**, *49*, 603–614. DOI: 10.1016/j.jddst.2018.12.027.
7. Jarvis, B.; Simpson, K. Clopidogrel: a review of its use in the prevention of atherothrombosis. *Drugs* **2000**, *60* (2), 347–377. DOI: 10.2165/00003495-200060020-00012.
8. Lassoued, M. A.; Sfar, S.; Bouraoui, A.; Khemiss, F. Absorption enhancement studies of clopidogrel hydrogen sulphate in rat everted gut sacs. *J. Pharm. Pharmacol*. **2012**, *64* (4): 541–552. DOI: 10.1111/j.2042-7158.2011.01434.x
9. Bali, D. E.; Osman, M. A.; El Maghraby, G. M. Enhancement of dissolution rate and intestinal stability of clopidogrel hydrogen sulfate. *Eur. J. Drug Metab. Pharmacokinet*. **2016**, *41* (6), 807–818. DOI: 10.1007/s13318-015-0311-4.
10. El-Laithy, H. M.; Badawi, A.; Abdelmalak, N. S.; Elsayyad, N. M. E. Stabilizing excipients for engineered clopidogrel bisulfate procubosome derived in situ cubosomes for enhanced intestinal dissolution: stability and bioavailability considerations. *Eur. J. Pharm. Sci*. **2019**, *136*, 104954. DOI: 10.1016/j.ejps.2019.06.008.
11. Sonje, V. M.; Kumar, L.; Meena, C. L.; Kohli, G.; Puri, V.; Jain, R.; Bansal, A. K.; Brittain, H. G. Atorvastatin Calcium. In *Profiles of Drug Substances, Excipients and Related Methodology*; Elsevier, 2010; Vol. 35, pp 1–70. DOI: 10.1016/S1871-5125(10)35001-1.
12. Lestari, M. L. A. D.; Suciati; Indrayanto, G.; Brittain, H. G. Clopidogrel bisulfate. In *Profiles of Drug Substances, Excipients and Related Methodology*; Elsevier, 2010; Vol. 35, pp 71–115. DOI: 10.1016/S1871-5125(10)35002-3.
13. Alam, S.; Patel, S.; Bansal, A. K. Effect of sample preparation method on quantification of polymorphs using PXRD. *Pharm. Dev. Technol*. **2010**, *15* (5), 452–459. DOI: 10.3109/10837450903286511.

14. Reflection paper on the dissolution specification for generic solid oral immediate release products with systemic action. EMA/CHMP/CVMP/QWP/336031/2017; Committee for Medicinal Products for Human use (CHMP), Committee for Medicinal Products for Veterinary use (CVMP), Quality Working Party (QWP), European Medicines Agency, August 10, 2017.
15. Buffer solutions. In *The United States Pharmacopeia and National Formulary USP 41–NF 36*; The United States Pharmacopeial Convention, Inc.: Rockville, MD, 2020.
16. Moore, J. W.; Flanner, H. H. Mathematical comparison of curves with an emphasis on in vitro dissolution profiles. *Pharm. Technol*. **1996**, *20*, 64–74.
17. El Orche, A.; Bouatia, M.; Yanisse, S.; Labjar, H.; Mouhsin, M.; Bouha, M.; Oubenali, M.; Mbarki, M. Evaluation of the capability of horizontal ATR-FTMIR and UV-visible spectroscopy in the discrimination of virgin olive oils from the Moroccan region of Beni Mellal-Khenifra. *J. Spectrosc*. **2020**, *2020*, 1–9. DOI: 10.1155/2020/9317350.
18. Bro, R.; Smilde, A. K. Principal component analysis. *Anal. Methods* **2014**, *6* (9), 2812–2831. DOI: 10.1039/C3AY41907J.
19. Maggio, R. M.; Castellano, P. M.; Kaufman, T. S. A new principal component analysis-based approach for testing "similarity" of drug dissolution profiles. *Eur. J. Pharm. Sci*. **2008**, *34* (1), 66–77. DOI: 10.1016/j.ejps.2008.02.009.
20. Chelcea, S. Agglomerative 2-3 Hierarchical Classification: Theoretical and Applicative Study. **2007**, 241. https://tel.archives-ouvertes.fr/tel-00156809
21. Stanimirova, I.; Daszykowski, M. Exploratory analysis of metabolomic data. In *Comprehensive Analytical Chemistry*; Jaumot, J., Bedia, C., Tauler, R., Eds.; Elsevier, 2018; Vol. 82, pp 227–264. DOI: 10.1016/bs.coac.2018.08.005.
22. Gavioli, A.; de Souza, E. G.; Bazzi, C. L.; Schenatto, K.; Betzek, N. M. Identification of management zones in precision agriculture: an evaluation of alternative cluster analysis methods. *Biosyst. Eng*. **2019**, *181*, 86–102. DOI: 10.1016/j.biosystemseng.2019.02.019.
23. Gary Hollenbeck, R.; Mitrevej, K. T.; Fan, A. C. Estimation of the extent of drug-excipient interactions involving croscarmellose sodium. *J. Pharm. Sci*. **1983**, *72* (3), 325–327. DOI: 10.1002/jps.2600720333.
24. Zarmpi, P.; Flanagan, T.; Meehan, E.; Mann, J.; Fotaki, N. Biopharmaceutical understanding of excipient variability on drug apparent solubility based on drug physicochemical properties. Case study: superdisintegrants. *AAPS J*. **2020**, *22* (2), 46. DOI: 10.1208/s12248-019-0406-y.