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Determination of bioequivalence between generic and reference drugs using laser-induced breakdown spectroscopy

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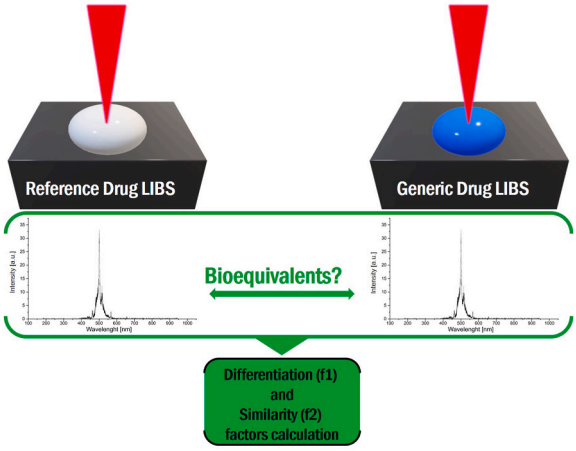
HIGHLIGHTS

- For the first time, the LIBS technique is used in an advanced pharma application.
- Drugs with a nominal content of 1 mg/tablet can be analysed by LIBS.
- To statistically compare LIBS spectra, the f1 and f2 factors are introduced.
- LIBS is used to accurate predict the generic drugs *in vitro* bioequivalence.

GRAPHICAL ABSTRACT



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ABSTRACT

Background: In vitro bioequivalence studies are strictly limited to the comparison of dissolution performance to a reference drug. These studies are performed without considering the chemical similarity between the generic and reference drug formulations. This work has focused on developing a groundbreaking method based on the laser-induced breakdown spectroscopy (LIBS) technique for the *in vitro* bioequivalence determination of immediate-release solid oral dosage form generic drugs and as an alternative method for establishing the biowaiver of *in vivo* generic drug studies.

Results: The novel LIBS-based methodology to determine *in vitro* bioequivalence is fast, easy to perform, and can be carried out without the requirement of tedious and complicated sample pre-treatment, nor expensive instrumentals and reagents, almost directly on the drug samples. Furthermore, the proposed methodology demonstrated that it is enough to identify the spectrochemical similarity of the formulation between generic drugs to a reference drug through the chemometric study of their LIBS spectra, based on the determination of the

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differentiation and similarity factors, f_1 and f_2, respectively, used in the pharmaceutical industry in this purpose. After analysing their LIBS spectra, the generic drugs selected for this work have all been shown to be *in vitro* bioequivalent, given their f_1 values of less than 15 and f_2 values greater than 50, according to the technical regulations on which the American and European medicines agencies are based for the approval of registration for generic immediate-release solid oral dosage form drugs. This has been evidenced even for drugs from Class III and Class IV of the biopharmaceutical classification system, whose active principle nominal concentration is very low as 0.1 and 0.25 mg/tablet, respectively.

Significance: for the first time the LIBS technique has been successfully used in an advanced application for the pharmaceutical industry. The proposed method constitutes a reliable and specialized methodology for the establishment of formulation similarity between two drugs, without the requirement of separate identification of each of their components, which is a new and potential tool to determine the *in vitro* bioequivalence for generic immediate-release solid oral dosage form drugs.

1. Introduction

According to the United States Food and Drug Administration (USFDA), bioequivalence is defined as "the absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study" [1]. Usually, a generic immediate-release solid oral dosage form drug can demonstrate bioequivalence using *in vivo* or *in vitro* procedures [1,2]. In the first case the pharmacokinetic performance of the drug is tested in patients through bioavailability studies. For the second case, the bioequivalence is determined by studying the difference and similarity between the dissolution profiles of the generic drug and the reference drug, using mathematical algorithms expressly indicated and approved by drug regulatory agencies [1–4]. Specifically, *in vitro* bioequivalence studies involve the quantitative comparison of the difference and similarity between the dissolution profile over time of two drugs, one generic and one reference drug, i.e. the quantification of whether the two drugs dissolve similarly. Naturally, this particular kind of pharmaceutical study entails a tremendous cost for the pharmaceutical industry, which translates into an increase in the cost of generic drugs for the consumer. In addition, these studies require specialized personnel and sophisticated instruments, which implies a study of a very complex scope that can take significant time to complete execution [5]. Specifically, *in vitro* bioequivalence studies involve the use of advanced analytical techniques, such as dissolution apparatus or "dissolutors" coupled on-line with UV/vis spectrometers, liquid chromatography with diode array detector (HPLC-DAD), among others, which in turn require the use of reagents and solvents that, in addition to being expensive, involve the use of reagents and other chemical wastes that needs further treatment procedures that constitute a risk to human and environmental health, not to mention the specialized consumables required in the sample treatment and analytes separation stages [5,6].

Laser-induced breakdown spectroscopy (LIBS) is an analytical technique with a wide field of application in the analysis of several samples such as geomaterials [7], foods [8–11], water [12–14], and even for biological samples [15–18] and bacteria [19–21], among many other samples for advanced applications [22]. This broad application scope is due to the ability of this technique to produce elemental fingerprint spectra of any pure substance or mixtures of them [23–25] from the emissions of the various atoms that make up the components of a sample associated with its unique chemical composition [26,27], either from a qualitative or quantitative objective or both. Although the analytical suitability of the LIBS technique has been demonstrated for use in routine pharmaceutical applications, both qualitative [28] and quantitative [29], the current reality is that LIBS-based applications are required to solve more sophisticated and challenging analytical pharmaceutical problems. Since it is possible to obtain unique and characterized elemental fingerprint spectra for each drug [27–29], LIBS-based analysis is a tool of particular interest in the field of pharmaceutical quality control, most especially for analysis at drug development stages, such as drug identification, and drug adulteration or fraud; since LIBS is widely and especially used for these purposes at present, but not on pharmaceutical samples. However, the development of pharmaco-analytical applications based on LIBS for the advanced study of drugs is a niche of growing interest, given the technical and instrumental advantages of this type of analytical spectroscopy.

This work has focused on the development of a new analytical alternative for the determination of the *in vitro* bioequivalence of immediate-release oral solid dosage forms using the LIBS technique, for the first time used in this type of advanced drug-related applications, and the introduction of the statistical algorithms similarity and differentiation factors, used by the pharmaceutical industry on *in vitro* dissolution studies, as a new method for the comparison of LIBS spectra.

2. Material and methods

2.1. Drug samples and preliminary preparations

Commercially available reference and generic drugs samples of the same active ingredient were selected to develop this work. Table 1 details the information on the samples used. A total of 41 different medicines have been analysed, of which 16 are reference medicines and 25 are generic pharmaceutical equivalents. Overall, this study has been carried out using 16 different medicines, corresponding to different categories of the biopharmaceutical classification system (BCS). Specifically, 4 drugs from category I, 3 drugs from category II, 4 drugs from category III and 5 drugs from category IV were used. The purpose of the study was to validate the capability of the proposed method for drugs belonging to different BCS categories, i.e. drugs with different solubility and permeability characteristics. A total of 41 different medicines have been analysed, of which 16 are reference medicines and 25 are generic pharmaceutical equivalents.

To verify the suitability of the LIBS technique for the analytical study of drugs over a wide range of nominal active substance content, the drugs were carefully selected with a nominal concentration ranging between 0.25 and 850 mg. All used formulations were film-coated immediate-release tablets, and all the generic tested drugs have the Generic Pharmaceutical Equivalent (EFG) denomination. This means that they are equivalent to the branded originals, containing the same active ingredients, the same pharmaceutical presentation, and offering the same quality, safety, and efficacy; they are also reliable medicines since their reference standard is the branded originals, which have been on the market for at least 10 years, and their therapeutic efficacy has been fully demonstrated by the rigorous bioequivalence studies [30–32]. A minimum of one generic drug samples by each reference drug were used in this study. For each drug, the average mass of 20 tablets was determined, which were later pulverized with the help of a porcelain mortar. Subsequently, the powder was transferred to an electric mill to ensure homogeneity in the particle size of the powder. The powder was then passed through a 100 μm mesh. Finally, tablets were prepared from the powder, with a mass equal to the original tablets' average ($n = 20$) mass.

2.2. Instrumental set-up and sample analysis

The LIBS technique principles, and the methodology used in the present work, and the most significant experimental conditions have been previously described [33]. Thus, only the experimental conditions relevant to this study are presented here. Briefly, LIBS measurements were obtained using a Q-switched Nd:YAG laser at 1064 nm (Brio, Quantel), with a 10 Hz frequency setting. The laser pulse duration, energy by pulse, time delay, gate width, and spot size were four ns, 20 mJ/cm^2, 2μs, 1 ms, and 0.1 mm, respectively. Plasma optical emission was analysed between 200 and 950 nm with a two-channel AvaSpec DUAL spectrometer (Avantes) and resolutions of 0.08 and 0.17 nm, respectively. The measurements were taken under room conditions. Fifty spectra were obtained for each drug sample by striking them in different places on their surface. The spectra were processed using AvaSoft software version 8.3.1.0 (2015) (Avantes).

2.3. Statistical treatment for generic and reference drug LIBS spectrum

All obtained LIBS drug spectra were baseline corrected by applying asymmetrically reweighted penalized least squares smoothing (arPLS) [8] using MATLAB R2022a. After this, each target drug's average spectrum (n = 50) was calculated. Then, to purge each drug's spectral dataset of aberrant spectra, Pearson's linear correlation coefficient and Spearman's nonparametric rank coefficient [11] were estimated for every 50 individual spectra relative to the average spectrum and fitted to a probability distribution function. Thus, Pearson's linear correlation coefficients were fitted to a beta distribution function, whereas Spearman's nonparametric rank coefficients were fitted to a Weibull distribution function. The probability distribution fit was measured using Statgraphics Centurion 19.1.2 (Statgraphics Technologies Inc.). Given a confidence level of 70 %, spectra with Pearson's and Spearman's coefficients outside this confidence level were discarded. In general terms, 10–20 % of the original 50 spectra were removed, i.e. around 5–10 spectra for each of the samples analysed. From the remaining spectra, a new average spectrum was calculated from which the differentiation and similarity factors were estimated.

2.4. In vitro determination of the bioequivalence of generic immediate-release solid oral dosage form drugs by LIBS

First, for a preliminary comparison of the LIBS spectral similarity of the reference drugs and their associated generic, the correlation of the spectra for each generic spectrum was studied as a function of the drug reference spectrum. For this purpose, the complete spectral information of each spectrum was used, i.e. all the obtained intensities from 200 to 950 nm (9296 wavelength and intensity points), to generate a dispersion graph of the intensities obtained for each wavelength that constitutes the spectra. As a preliminary measure to estimate the spectral similarity or correlation between the generic and reference spectra of the drugs studied, Pearson's coefficient was calculated for each of the 16 drugs studied. After this, the average spectrum of the generic drugs was statistically compared with the reference drug spectrum using the difference (f_1) and similarity (f_2) factors applied to *in vitro* bioequivalence

Table 1
Drug samples information.

Active Principle	Chemical Formulae	Nominal Concentration	BCS Category	Manufacturer	Type of Drug
Paracetamol	$C_8H_9NO_2$	650 mg	I	Ferrer	Reference
				Kern Pharma	Generic
				Normon	Generic
Acetylsalicylic acid	$C_9H_8O_4$	100 mg	I	Bayer	Reference
				Cinfa	Generic
				Kern Pharma	Generic
Lorazepam	$C_{15}H_{10}Cl_2N_2O_2$	1 mg	I	Pfizer	Reference
				Cinfa	Generic
				Kern Pharma	Generic
Alprazolam	$C_{17}H_{13}ClN_4$	0.25 mg	I	Viatris	Reference
				Cinfa	Generic
				Sandoz	Generic
Diclofenac sodium	$C_{14}H_{10}Cl_2NNaO_2$	100 mg	II	Novartis	Reference
				Pensa	Generic
Simvastatin	$C_{25}H_{38}O_5$	10 mg	II	Lacer	Reference
				Sandoz	Generic
				Kern Pharma	Generic
Finasteride	$C_{23}H_{36}N_2O_2$	1 mg	II	Cantabria Labs	Reference
				Kern Pharma	Generic
Metformin	$C_4H_{11}N_5$	850 mg	III	Merck	Reference
				Cinfa	Generic
				Sandoz	Generic
Amoxicillin	$C_{16}H_{19}N_3O_5S$	500 mg	III	GSK	Reference
				Cinfa	Generic
				Teva	Generic
Lisinopril	$C_{21}H_{31}N_3O_5$	5 mg	III	Neuraxpharm	Reference
				Ratiopharm	Generic
Levothyroxine sodium	$C_{15}H_{10}I_4NNaO_4$	0.1 mg	III	Merck	Reference
				Sanofi	Generic
Cefuroxime	$C_{16}H_{16}N_4O_8S$	250 mg	IV	GSK	Reference
				Cinfa	Generic
				Normon	Generic
Furosemide	$C_{12}H_{11}ClN_2O_5S$	40 mg	IV	Sanofi	Reference
				Kern Pharma	Generic
				UXA Pharma	Generic
Haloperidol	$C_{21}H_{23}ClFNO_2$	5 mg	IV	Esteve	Reference
				Kern Pharma	Generic
Methotrexate	$C_{20}H_{22}N_8O_5$	2.5 mg	IV	Wyeth	Reference
				Cipla	Generic
Digoxin	$C_{41}H_{64}O_{14}$	0.25 mg	IV	Teofarma	Reference
				Kern Pharma	Generic

studies of generic immediate-release solid oral dosage form drugs [1,2,4], being adapted to the spectrochemical comparison. Thus, wavelength and signal intensity were considered parameters instead of time and dilution percentage.

$$f_1 = \left\{ \frac{\sum_{i=1}^{n} |R_i - T_i|}{\sum_{i=1}^{n} R_i} \right\} \times 100 \quad (1)$$

$$f_2 = 50\, log\left\{ \left(1 + \frac{1}{n} \sum_{i=1}^{n} (R_i - T_i)^2 \right)^{-0.5} \times 100 \right\} \quad (2)$$

Where R_i is the signal intensity for a given wavelength of the reference drug spectrum, T_i is the signal intensity at the same wavelength of the generic drug spectrum, and n refers to the total number of wavelength positions of the obtained LIBS spectra, that is, 7296 positions that make up each spectrum. For the calculation of the values of the factors f_1 and f_2, the guidelines established by the USFDA [34] and European Medicines Agency (EMA) [2] were followed:

(1) All LIBS measurements were made under the same experimental conditions; (2) Twenty individual dosage units were used for the generic and reference drug; (3) the coefficient of variation of either drug was less than 5 % for each wavelength.

3. Results and discussion

3.1. Reference and generic drug LIBS spectrum

LIBS technique allows obtaining a characteristic spectrum or fingerprint associated with the elemental composition of the drug analysed with high sensitivity. The average spectrum of the generic and reference for each analysed drug are shown in Fig. 1.

At first glance, the spectral peculiarities for each of the 16 different drugs analysed in this study can be observed using the LIBS technique. Since each drug is composed of a characteristic active ingredient, in addition to its excipients, characteristic and differentiated spectra are observed for each drug analysed. On the other hand, when visually analysing the LIBS spectra for each reference drug and its respective generic(s), a coincidence in the emission lines for each drug is observed, which is attributed, in principle, to the similarity between the formulations that constitute the reference and generic samples, for each drug studied. These results evoke the potential use of LIBS spectra for the identification of elemental fingerprints associated with drugs, as has been previously evidenced for foods [35], geomaterials [7], bacteria [19], among other types of samples [14,16,36]. Although it is true that a variation in terms of emission line intensities is observed, this spectral behaviour is associated with differences in the actual concentration of the active ingredient in the drugs. In pharmacopoeial terms and depending on the active ingredient, the actual concentration of a drug can vary between 5 and 20 % of the nominal concentration declared by the manufacturer [37–39]. Even according to pharmacopoeial standards, variations between 5 and 10 % in content uniformity are allowed [37–39], which explains the analytical impossibility of obtaining exactly equal spectra, in terms of the intensities of the emission lines that make up the spectrum, for drugs of the same active ingredient and nominal concentration, manufactured under the same conditions.

As shown in Table 1, the drugs studied involve organic molecules consisting mostly of C, H and O. Additionally, the element N and S are also constituents of some drugs, in addition to the cases where the halogens Cl, I and F are present in the molecule of some studies drugs. In a generalised way, we can derive this constitution of active ingredients from the LIBS spectrum, using the NIST Atomic Spectra Database [40] and the NIST LIBS database [41], both available online. For all the spectra obtained for the drugs, the main emission lines of C I ($2s^2 2p4p$ - $2s^2 2p3s$) are present at 505.21 nm, H I (3d - 2p) at 656.27 nm, and O I ($2s^2 2p^3(4\,S^0)3p$ - $2s^2 2p^3(4\,S^0)3s$) at 777.41 nm. In the case of the spectra of paracetamol (Fig. 1a), acetylsalicylic acid (Fig. 1b), lorazepam (Fig. 1c) and simvastatin (Fig. 1f), where the O I emission line at 777.41 nm is not evident, the O II ($2s^2 2p^2(3P)3d$ - $2s^2 2p^2(3P)3p$) line is observed at 515.99 nm. This is because both paracetamol and acetylsalicylic acid are manufactured by direct compression, i.e. without the use of any excipients; and in the case of simvastatin and lorazepam, because of the low active ingredient concentration of 1 mg and 10 mg, respectively.

In the case of medicines with the element nitrogen in the molecule of their active ingredient, such as paracetamol (Fig. 1a), lorazepam (Fig. 1c), alprazolam (Fig. 1d), diclofenac sodium (Fig. 1e), finasteride (Fig. 1g), Metformin (Fig. 1h), amoxicillin (Fig. 1i), levothyroxine sodium (Fig. 1k), cefuroxime (Fig. 1l), furosemide (Fig. 1m), and methotrexate (Fig. 1o); the emission lines of N III ($2s^2 3p$ - $2s^2 3s$) and N I ($2s^2 2p^2(1d)3p$ - $2s^2 2p^2(3p)3s$) are observed at 410.34 nm and 410.99 nm, respectively. In the specific situation of the drugs lisinopril (Fig. 1j) and haloperidol (Fig. 1n) whose active substance molecule contains N but whose N III and N I lines are not evident in their LIBS spectra, the N III line ($2s2p(3p^0)3p$ - $2s2p(3p^0)3s$) is observed at 419.58 nm. For drugs whose active ingredient is also composed of the S-element, specifically amoxicillin (Fig. 1i), cerufoxime (Fig. 1l) and furosemide (Fig. 1m), the S I emission lines ($3s^2 3p^3(4\,S^0)5p$ - $3s^2 3p^3(4\,S^0)4s$) are observed at 469.54 nm. The Cl I emission line ($3s^2 3p^4(3p)5p$ - $3s^2 3p^4(3p)4s$) at 443.85 nm can be observed in the spectra of the drugs lorazepam (Fig. 1c), alprazolam (Fig. 1d), diclofenac sodium (Fig. 1e), furosemide (Fig. 1m) and haloperidol (Fig. 1n), whose active principle is made up of the chlorine element. The LIBS spectrum of levothyroxine sodium (Fig. 1k), the only drug studied with iodine in its molecular composition, shows the I II emission line ($5s^2 5p^3(4s^0)6p$ - $5s^2 5p^3(4s^0)6s$) at 595.02 nm. This result constitutes an important milestone in the use of the LIBS technique and an evidence of the analytical selectivity and sensibility for the detection of I in drugs, given that the nominal concentration of the active ingredient in the tablet is only 0.1 mg, which corresponds a nominal content of 63.5 μg of I per tablet. A similar result was obtained for the F II emission line ($2s^2 2p^3(2\,d^0)3p$- $2s^2 2p^3(2\,d^0)3s$) observed at 429.92 nm, present in the spectrum of haloperidol (Fig. 1n), the only drug used in this study with fluorine in its chemical composition. Finally, the emission lines of Na I ($2p^6 3p$ - $2p^6 3s$) at 589.14 nm can be observed in the spectra of diclofenac sodium (Fig. 1e) and levothyroxine sodium (Fig. 1k), the only drugs used in this study with sodium in their chemical composition. In addition to the emission signals described above, the spectra of the drugs studied may show multiple signals of other elements outside the chemical composition of the active substance due to the elemental contribution coming from the elements present in the excipients which, together with the active substance, constitute the magistral formula of a drug. All these results are consistent with previous studies where LIBS has been used for the detection of elemental species in drugs [28,29,42,43].

3.2. Reference and generic drugs spectral correlations

The results of the study of the correlation between generic and reference drugs are shown in Fig. 2. For all groups of generic and reference drug cases, the correlation factor was higher than 0.75, except for lorazepam Kern Pharma, which had a 0.7073 with respect to the reference drug; stating the high spectrochemical correlation between the LIBS spectrum of generic drugs and the reference drug.

In the specific case of establishing the correlation between the spectra of drugs with different active principle and drugs with the same active principle and different nominal concentration of active principle, no case presented a correlation factor greater than 0.45, indicating a low correlation between the LIBS spectra for these two cases. This demonstrates the analytical efficiency of LIBS spectra as a unique elemental fingerprint for each sample, even for drugs with the same active principle and different nominal concentration.

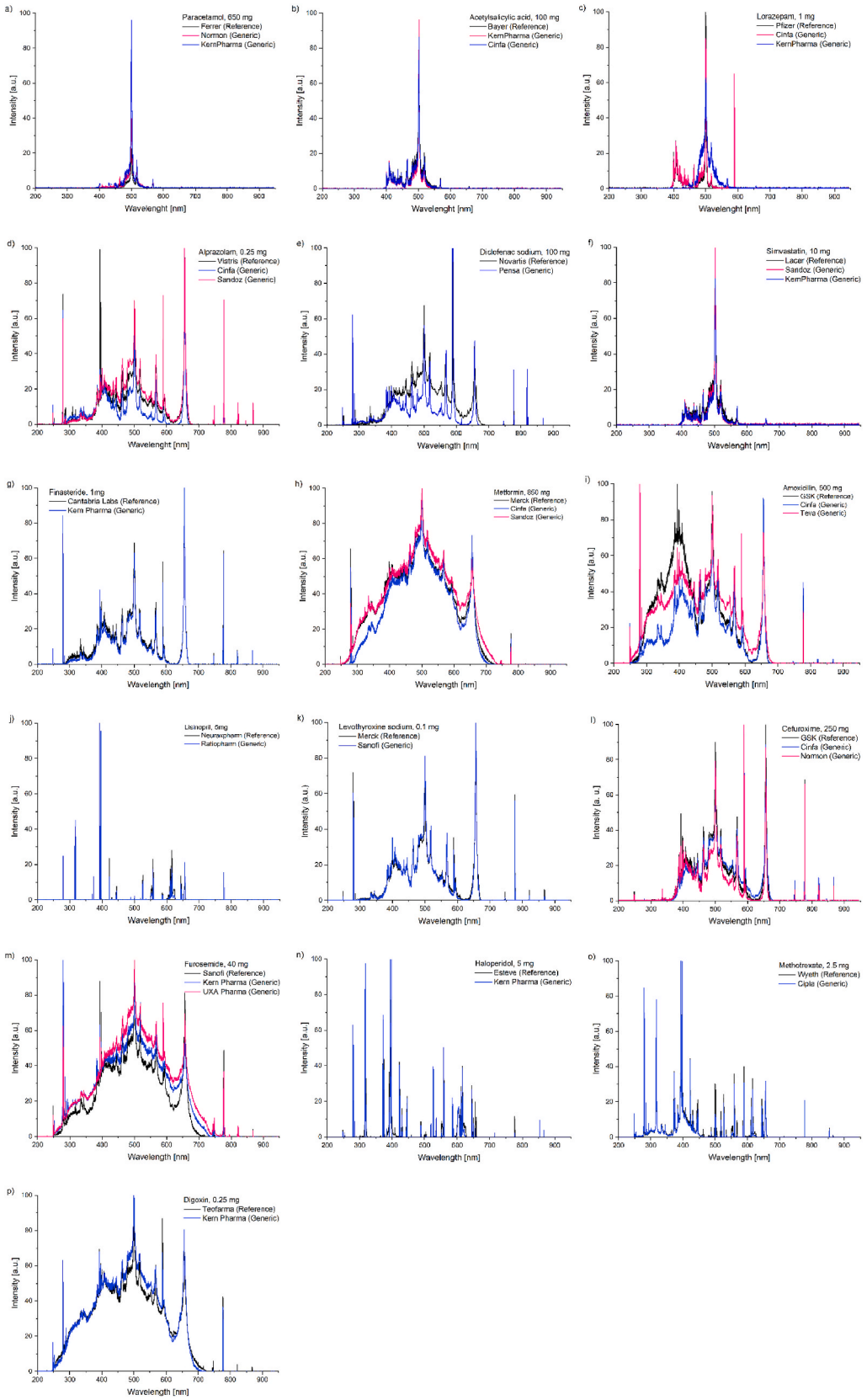

Fig. 1. Typical LIBS spectrum of the drugs evaluated in this study. a) Paracetamol; b) Acetylsalicylic acid; c) Lorazepam; d) Alprazolam; e) Diclofenac sodium; f) Simvastatin; g) Finasteride; h) Metformin; i) Amoxicillin; j) Lisinopril; k) Levothyroxine sodium; l) Cefuroxime; m) Furosemide; n) Haloperidol; o) Methotrexate; and p) Digoxin.

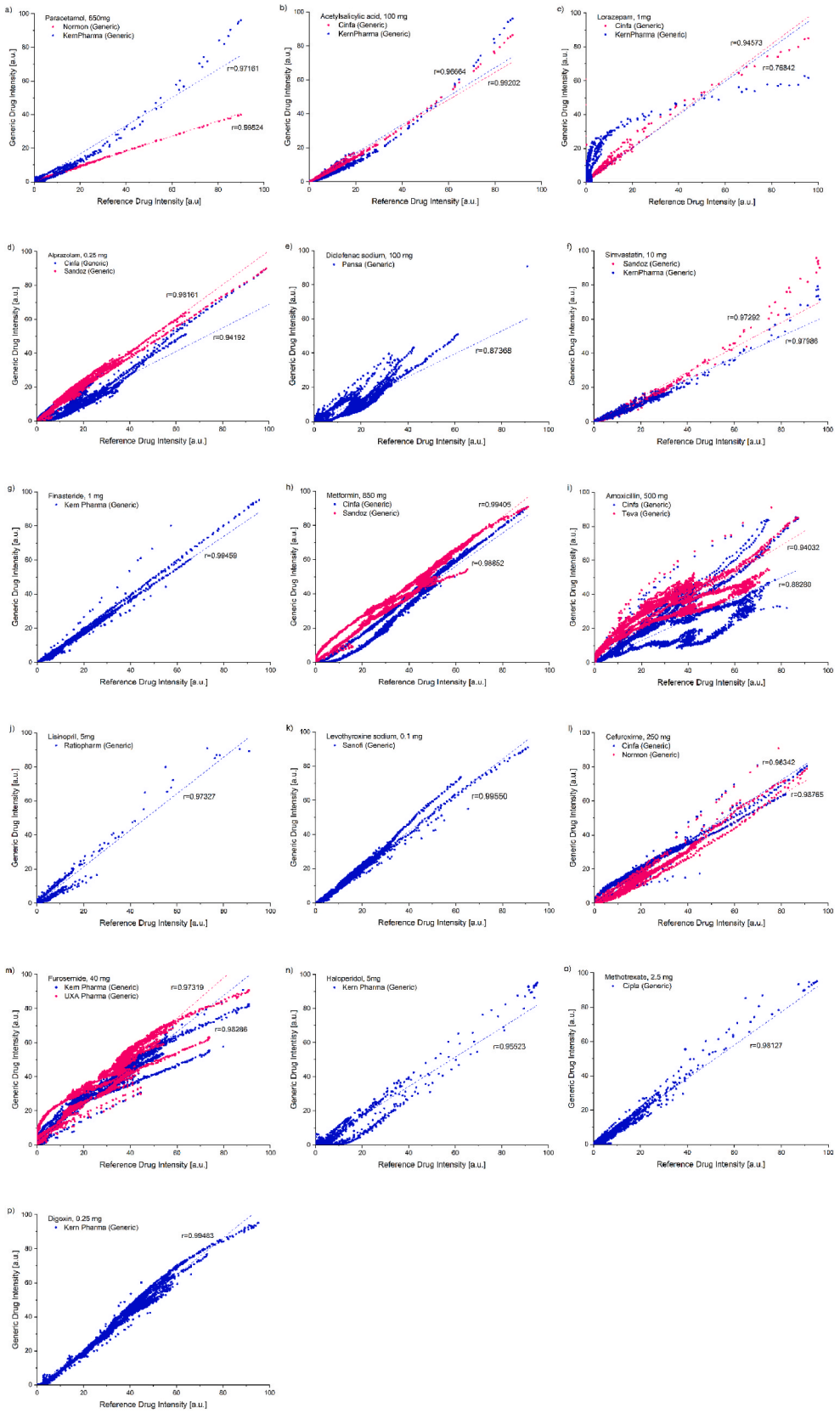

Fig. 2. LIBS spectrum correlation of generic and reference drugs. 1a) Paracetamol; b) Acetylsalicylic acid; c) Lorazepam; d) Alprazolam; e) Diclofenac sodium; f) Simvastatin; g) Finasteride; h) Metformin; i) Amoxicillin; j) Lisinopril; k) Levothyroxine sodium; l) Cefuroxime; m) Furosemide; n) Haloperidol; o) Methotrexate; and p) Digoxin.

3.3. Reference and generic drug spectrum similarity

The differentiation (f_1) and similarity (f_2) factors have been adopted by the USFDA (1997) [34] and the European Agency for the Evaluation of Medicinal Products [2] by the Committee for Medicinal Products (CPMP) to compare the dissolution profile [4]. Two dissolution profiles, from generic and reference drugs are considered bioequivalent if f_1 ranges from 0 to 15 and f_2 ranges from 50 to 100 [2,4,34]. Since these studies are conducted under *in vitro* conditions, i.e., in a controlled environment outside the human body, the dissolution of the generic drug is strictly dependent on its formulation [4,44–48], so it involves a comparison of the performance of two formulations, one generic and one reference drug. In this sense, the comparative study of formulations between two drugs, one generic and one reference drug, is a potential tool for determining bioequivalence under these conditions. So basically, the more similar the formulation, the greater the similarity between the dissolution of a generic drug and a reference drug. In other words, the greater it is *in vitro* bioequivalence, in line with previous studies [49]. This criterion was applied to establish the similarity of the drug's formulation based on the spectrochemical studies of the LIBS spectra of generic drugs concerning their reference drugs, using the previously described algorithms established by USFDA and EMEA. Table 2 shows the differentiation and similarity factors obtained for each generic drug with respect to its reference drug.

According to the results obtained, the generic drugs, for each case of active principle, met the criteria of $0<f_1<15$ % and 50 %$<f_2<100$ %, so it is possible to infer that their formulations are very similar. Therefore, given the similarity of the LIBS spectra of each one, they will have the same dissolution performance since if the spectra are similar, the formulations are also similar. However, it is important to remember that the LIBS spectra obtained for each drug constitute a unique fingerprint for each drug, and yes and only yes, two LIBS spectra will be similar if the chemical composition of the substances from which they were obtained are similar.

These results of the determination of the f_1 and f_2 factors from the LIBS spectral information of the studied drugs are important evidence to justify that all tested generic drugs are bioequivalent with respect to their corresponding reference drugs. In other words, the generic drugs will exhibit similar dissolution performance, so technically, they can be considered bioequivalent drugs. Since this methodology has been developed for the *in vitro* bioequivalence study of generic immediate-release solid oral dosage form drugs, it can easily be applied for capsules, using the capsule contents similarly as stated in section 2.1. Drug Samples and preliminary preparations.

The development of this work could constitute the framework for introducing a new technical criterion strictly related to the qualitative-quantitative formulation of drugs for obtaining bioequivalence of generic drugs through spectrochemical studies with LIBS, constituting an alternative approach to assessing bioequivalence [50]. This would imply a tremendous and vital advance in generic drug production. It significantly minimizes the time, resources (instrumental and economic), and complexity in executing *in vitro* bioequivalence studies. This translates into safer generic drugs, expanding the range of generics on the market and cheaper drugs since R&D resources are significantly reduced. Perhaps another impact of this research is that this novel approach could be applied as a biowaiver criterion to APIs of category II and IV of the Biopharmaceutical Classification System, avoiding the need to obligatorily carry out *in vivo* bioavailability studies for generic drugs that their APIs belong to these BCS categories [51–53]. On the other hand, the use of statistical algorithms for the comparison of LIBS spectra, oriented to discern the spectral similarity quantitatively and differentiation of two samples, allows contributing to the lines of research aimed at the development of LIBS spectral libraries and the identification of substances from them, based on the use of the statistical treatments that have been used in this research.

Table 2
Differentiation (f_1) and similarity (f_2) factors of generic drugs obtained by LIBS.

Drug Denomination		Bioequivalence Factors	
Reference	Generic	f_1	f_2
Ferrer Paracetamol (Gelocatil) 650 mg	Kern Pharma Paracetamol 650 mg	5.51	98.92
	Normon Paracetamol 650 mg	4.75	99.34
Bayer Acetylsalicylic acid (Adiro) 100 mg	Kern Pharma Acetylsalicylic acid 100 mg	10.80	89.03
	Cinfa Acetylsalicylic acid 100 mg	9.89	90.2
Pfizer Lorazepam (Orfidal) 1 mg	Cinfa Lorazepam 1 mg	9.89	91.57
	Kern Pharma Lorazepam 1 mg	12.41	72.98
Viatris Alprazolam 0.25 mg	Cinfa Alprazolam 0.25 mg	4.15	93.02
	Sandoz Alprazolam 0.25 mg	6.76	89.31
Novartis Diclofenac Sodium (Voltarén) 100 mg	Pensa Diclofenac sodium 100 mg	7.74	81.07
Lacer Simvastatin (Pantok) 10 mg	Sandoz Simvastatin 10 mg	10.67	79.69
	Kern Pharma Simvastatin 10 mg	8.62	95.18
Cantabria Labs Finasteride (Alocare) 1 mg	Kern Pharma Finasteride 1 mg	2.77	97.01
Merck Metformin HCl (Dianben) 850 mg	Cinfa Metformin HCl 850 mg	5.83	84.49
	Sandoz Metformin HCl 850 mg	2.18	92.62
GSK Amoxicillin (Amoxil) 500 mg	Cinfa Amoxicillin 500 mg	11.38	76.79
	Teva Amoxicillin 500 mg	7.19	89.06
Neuraxpharm Lisinopril (Doneka) 5 mg	Ratiopharm Lisinopril 5 mg	2.06	98.53
Merck Levothyroxine sodium (Eutirox) 0.1 mg	Sanofi Levothyroxine sodium 0.1 mg	1.48	97.95
GSK Cerufoxime (Zinnat) 250 mg	Cinfa Cefuroxime 250 mg	3.60	94.16
	Normon Cefuroxime 250 mg	6.23	90.07
Sanofi Furosemide (Seguril) 40 mg	Kern Pharma Furosemide 40 mg	4.85	93.48
	UXA Pharma Furosemide 40 mg	6.33	86.16
Esteve Haloperidol 10 mg	Kern Pharma Haloperidol 10 mg	4.69	95.21
Wyeth Methotrexate 2.5 mg	Cipla Methotrexate 2.5 mg	2.35	96.94

(*continued on next page*)

Table 2 (*continued*)

Drug Denomination		Bioequivalence Factors	
Reference	Generic	f_1	f_2
Teofarma Digoxin 0.25 mg	Kern Pharma Digoxin 0.25 mg	4.10	88.39

4. Conclusions

For the first time, the LIBS technique was successfully applied to develop an advanced analytical application for pharmaceutical analysis, specifically for the assessment of the *in vitro* bioequivalence of generic immediate-release solid oral dosage form drugs, using the mathematical algorithms that dictate the bioequivalence regulations of the American (USFDA) and European (EMEA) medicines agencies. Also, the LIBS technique has generated specific spectra for each drug studied, even for those whose nominal concentration is as low as 1 mg/tablet, demonstrating its high sensitivity for detecting drug sample actives.

These results open a new perspective on using laser spectroscopy in pharmaceutical applications with a new, easy-to-perform method for the drug's bioequivalence and biowaiver testing, increasing the quantity and accuracy of the generic drug information. In this sense, the LIBS technique could be introduced as a new alternative for conducting *in vitro* bioequivalence studies. Additionally, introducing the similarity and difference factors as a statistic method for the spectrochemical study of LIBS spectra has been conclusive for the quantitative establishment of the similarity between the spectra of generic drugs and their reference. Furthermore, it has proven to be as specific as when it is usually used in comparing dissolution profiles. The results of this research are of great relevance for the pharmaceutical industry and drug regulatory agencies, not only because of its novel approach but also because of the confidence in its results, the ease of its execution, and its low cost, which in turn results in greater production capacity of generic drugs and, in addition, could constitute a new technical criterion to support biowaiver applications and facilitate and expedite the procedures for the sanitary registration of generic drugs that impacts the generic drug availability in the pharmaceutical market.

CRediT authorship contribution statement

J. Cardenas-Escudero: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **F. Navarro-Villoslada:** Writing – review & editing, Writing – original draft, Formal analysis. **G. Bellini:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **D. Galán-Madruga:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Formal analysis, Conceptualization. **J.O. Cáceres:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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.

Data availability

Data will be made available on request.

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