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A critical study on the application of the zero-crossing derivative spectrophotometry to the photodegradation monitoring of lacidipine

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Abstract

An extensive study on the application of the "zero-crossing" technique for the analysis of a binary mixture of the photosensitive drug lacidipine and its corresponding by-product by derivative spectrophotometry is described. The technique has been compared to either conventional and recently developed UV spectrophotometric procedures, including chemometric methods. The prediction ability of the different analytical techniques has been checked by using the first-order derivative spectra of drug and photoproduct in binary mixtures. Relative advantages and drawbacks have been discussed. The zero-crossing technique suffers from several limitations, mostly ascribed to the selection of suitable signals along slopes of the spectral curve, giving rise to low accurate and precise results. The mean recovery from the zero-crossing analysis was calculated to lie in the 95.1–98.4% range for lacidipine, and 91.2–118.9% for the photoproduct. Chemometric methods showed a greater prediction ability with a 101.4–103.0% and 96.3–98.4% recovery for drug and degradation product, respectively.
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Keywords: Derivative spectrophotometry; Lacidipine; Multivariate analysis; Photodegradation; Zero-crossing

1. Introduction

The success of spectrophotometry in the field of analytical chemistry depends mainly on the easy manipulation and interpretation of absorption spectra. On the other hand, the traditional methods, based on the use of a restricted number of signals, proved to be often unsatisfactory for the analysis of multicomponent mixtures, owing to their low accuracy and precision.

Several techniques for the elaboration of spectrophotometric data have been proposed with the aim at extracting a largest number of analytical information from spectra composed of unresolved bands. Undoubtedly, a major success was reached with the derivative elaboration of the absorbance curves, by plotting the first or a higher order mathematical derivatives of absorbance against wavelength ($dA/d\lambda$). Derivative spectrophotometry has been applied to many chemical systems, such as pharmaceuticals, foods, cosmetics and environmental samples, as extensively reported in two recent reviews [1,2], giving rise to accurate and precise analytical results. The successive development of the zero-crossing technique (ZCT) was accordingly due to the feature of derivative spectra to show signals with either positive and negative value. This technique exploits the signal crossing through the abscissa axis, for a given component of a mixture, to assign the absorbance value to remaining components. ZCT resulted particularly effective in the analysis of several complex mixtures, when a wide peaks overlapping was present in the corresponding zero-order spectrum [3–8].

However, suitable analytical signals are often placed on the peak shoulders or characterized by a too low absorbance. This could heavily limit the accuracy and precision of the method, as the low stability of such signals is well known. In fact, the absorbance uncertainty is much higher when the measurement is performed on a spectral shoulder instead of a maximum, where the reliability is good enough even along restricted wavelength range. Moreover, the use of signals with low absorbance, but still significant for ZCT, could also contribute to a higher degree of uncertainty.

An extensive statistical study on the data obtained by application of ZCT on a large number of samples was accordingly

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undertaken and the results were compared to those obtained by other spectrophotometric techniques.

The study herein described has been carried out on a binary mixture of lacidipine (LAC) and its photodegradation product (LACd). Such a system was characterized by a low content of LACd, so offering a possibility to check ZTC in such an extreme condition. LAC is an antihypertensive drug with dihydropyridine structure, acting by blocking L-type calcium channels. LAC is extremely sensitive to light, similarly to all other drugs belonging to the same chemical class [9–15]. The detailed photo-chemical degradation process of the drug has recently been reported in the literature [16].

The photodegradation experiment was performed following the ICH recommendations for the drug stability tests [17]. The analytical validity of ZCT was assessed by comparison with conventional UV methods based on the use of specific signals or advanced multivariate procedures such as multiple linear regression analysis (MLRA), principal component regression (PCR) and partial least squares (PLS).

2. Experimental

Light exposure was minimized throughout all operations because of light-sensitivity of the drug. The photodegradation procedure was entirely carried out in a room lit up with a 60 W red lamp.

2.1. Instruments

Absorbance and derivative spectra were recorded along the wavelength range of 190–450 nm, in a 10 mm quartz cell, by using a Perkin-Elmer Lambda 40P spectrophotometer, under the following conditions: scan rate 1 nm/s; time response 1 s; spectral bandwidth 1 nm. First-order derivative spectra were elaborated by the Savitzky–Golay algorithm with a $\Delta\lambda$ value fixed at 6 nm. UV Winlab 2.70.01 software (Perkin-Elmer) was used for spectral acquisition and elaboration.

Photodegradation study was performed in an irradiation cabinet "Suntest CPS+" (Heraeus, Italy), which follows ICH guideline about the light source characteristics. The irradiation system was equipped with a xenon lamp, which provides to produce an output similar to the D65. D65 is the internationally recognized standard for outdoor daylight as defined in ISO 10977. Samples were irradiated in a λ range between 300 and 800 nm with a 350 W/m^2 irradiation power, corresponding to a light dose of 21 kJ/min/m^2. A careful control of the temperature was gained by a cooling device, to keep the degradation rate independent of temperature. The temperature was throughout maintained at 21 °C.

2.2. Multivariate analysis software

"Spectrum Quant+" software (Version 4.10, Perkin-Elmer) was used for the development of the chemometric methods. This software is able to apply several regression methods for the multivariate analysis, including PCR and PLS. The software allows the optimization of the calibration model by a selection of spectral regions and the development of a validation procedure. The combination of this software with the Winlab acquisition software proved to be particularly useful, since it made possible an automatic elaboration of the spectra without any preliminary extraction of the spectral data.

Table 1
Calibration set for binary mixtures of LAC and its photodegradation product LACd (expressed as μg/ml)

Sample	LAC	LACd
1	4.916	0.000
2	9.832	0.000
3	19.665	0.000
4	29.498	0.000
5	39.330	0.000
6	4.916	0.510
7	9.832	0.510
8	19.665	0.510
9	29.498	0.510
10	39.330	0.510
11	4.916	2.040
12	9.832	2.040
13	19.665	2.040
14	29.498	2.040
15	39.330	2.040
16	4.916	5.100
17	9.832	5.100
18	19.665	5.100
19	29.498	5.100
20	39.330	5.100
21	4.916	10.200
22	9.832	10.200
23	19.665	10.200
24	29.498	10.200
25	39.330	10.200

2.3. Chemicals

Pure LAC was kindly supplied by GlaxoSmithKline S.p.A. (Verona, Italy). The photodegradation product was isolated by direct oxidation of LAC, by irradiating a 200 mg/ml hexane suspension of the drug with an UV lamp (280–360 nm; 30 W, at a distance of 30 cm) until the absorbance peak at 300 nm, due to LAC only, disappeared. After an irradiation time of 60 h, the solid was filtered and washed with pure hexane. The identity and purity grade of LACd was confirmed by NMR spectroscopy, GC–MS chromatography and UV spectrophotometry. The data obtained perfectly overlapped those reported in the literature [16].

Lacipil 4 and 6 mg (GlaxoSmithKline, Italy) pharmaceutical specialties were obtained commercially.

2.4. Calibration set

The calibration set was built following a full factorial design by preparing 25 standard binary mixtures of LAC and LACd in ethanol, distributed on five concentration levels, as listed in Table 1. Drug and photoproduct concentrations were in a range 4.92–39.33 μg/ml and 0–10.20 μg/ml, respectively. The calibration set was throughout used in the different methods to establish

mathematical relationships between products concentration and respective analytical signals.

The full factorial design herein adopted is perfectly balanced since each level of each concentration of the main component is studied for an equal number of times at each level of each other concentration of the second component. The presence of only two components in the studied system fully justified the adoption of this design.

2.5. Prediction set

A set of synthetic ethanol mixtures containing LAC and LACd in different ratios was prepared in order to apply the defined analytical methods. The prediction set, reported in Table 2, was constructed by a full factorial design with concentration values in the same range as that used for the calibration set, but arranged on four levels. This set was used to define statistical effectiveness of the methods in terms of accuracy and precision.

Table 2
Concentrations (μg/ml) of references solutions in the prediction set

Sample	LAC	LACd
1	9.832	0.510
2	19.665	0.510
3	29.498	0.510
4	39.330	0.510
5	9.832	2.040
6	19.665	2.040
7	29.498	2.040
8	39.330	2.040
9	9.832	5.100
10	19.665	5.100
11	29.498	5.100
12	39.330	5.100
13	9.832	10.310
14	19.665	10.310
15	29.498	10.310
16	39.330	10.310

2.6. Photodegradation tests

Photodegradation procedures were carried out in the Suntest cabinet by a constant irradiation between 300–800 nm wavelength range. Radiant power was set at 350 W/m^2, corresponding to an energy value of 21 kJ/min/m^2. This value was chosen since degradation follows a linear kinetics when exposition is kept to low enough values of radiant power, as suggested by preceding studies [16]. The inner temperature was fixed to 25 °C.

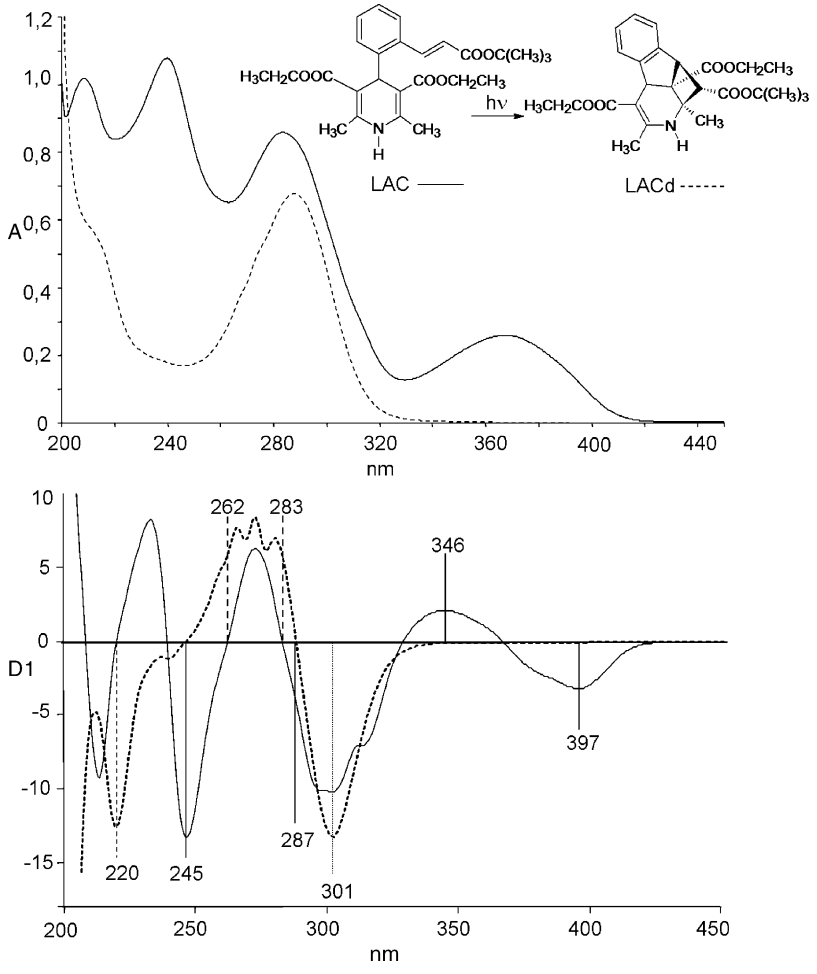

Fig. 1. Zero- and first-order derivative spectra of 20.64 μg/ml LAC (—) and 19.23 μg/ml LACd (– – –).

The photostability of the LAC pharmaceuticals was tested by exposing five tablets to artificial light and analysing them at different time intervals. For this aim, the tablets were weighed and reduced to a fine powder. An amount exactly corresponding to the average tablet weight was suspended in ethanol and the volume made up to 20 ml. The suspension was sonicated for 10 min and then filtered with a PTFE 0.45 μm membrane filter. 1 ml of this filtrate was diluted to 10 ml with ethanol and analyzed.

3. Results

All the LAC and LACd solutions were subjected to zero-to-fourth-order derivative spectrophotometric analysis. A deep examination of all the recorded spectra was done with the aim to select a suitable spectrum to be used for the simultaneous determination of the components by all the studied methods. A comparison study as more homogeneous as possible was so guaranteed.

Several specific signals were single out for each component in the various spectra but the first-order derivative spectra seemed to be the most suitable for the aim of this study. Zero- and first-order derivative spectra of 20.64 μg/ml drug and 19.23 μg/ml photoproduct, are reported in Fig. 1.

The spectra showed that the absorbance region over 340 nm was exclusively due to LAC and not influenced by LACd. Thus, the drug concentration was possible to be directly calculated by using a specific signal in this wavelength zone, being the absorption of the photoproduct negligible at these wavelengths. Further characteristic signals relative to a single component were pointed out at those wavelengths at which the spectrum of the other component crossed the *x*-axis. Both compounds showed finally a $^1D_{301}$ amplitude. This signal could be therefore used in a classical spectrophotometric procedure to determine the concentration of one component after having calculated its own absorbance contribution.

3.1. Classical derivative spectrophotometric methods

First-order derivative spectrum of the mixture showed a $^1D_{346–397}$ amplitude where the absorbance value was exclusively due to LAC. This absorbance value was never affected by the presence of LACd, at whatever concentration. The calibration relationship of LAC concentration against absorbance of $^1D_{346–397}$ was obtained by regression analysis, as reported in Table 3. LAC concentrations for the prediction set mixtures were calculated by using the above relationship. The statistical values in terms of overall accuracy and precision are summarized in Table 4.

In the same derivative spectrum both components showed an absorbance maximum at 301 nm. The total absorbance measured to this wavelength on several mixtures was verified to be exactly equal to the sum of absorbances of the two components. This signal can be used to calculate LACd concentration after subtracting the absorbance contribution of LAC. The LAC contribution at 301 nm can be in turn deducted from the ratio between the absorbance signals $^1D_{301}$ and $^1D_{346,397}$, which results constant for a LAC standard solution. $^1D_{346,397}$ absorbance can be directly measured from the mixture spectra since it is exclusively due to LAC and never influenced by LACd. The ratio between $^1D_{301}$ and $^1D_{346,397}$ was calculated as the average in a series of LAC standard solutions:

$$\frac{^1D_{301}}{^1D_{346,397}} = 1.837$$

Thus, LAC contribution in $^1D_{301}$ signal could be easily calculated by using this ratio value and the absorbance of the signal $^1D_{346–397}$. The absorbance contribution of LACd in $^1D_{301}$ signal was then deducted by subtracting LAC absorbance from the total. LACd concentration was eventually determined through the regression equation calculated from a series of LACd standard solutions, as reported in Table 3. The prediction results as recovery and RSD percentages are listed in Table 4.

Table 3
Calibration graphs for LAC and LACd (μg/ml) assay by classical and zero-crossing derivative spectrophotometry methods

λ	Analyte	Slope	Intercept	R^2
396–346	LAC	0.1432	−0.0160	0.9995
302	LACd	0.3191	−0.0346	0.9972
220	LACd	0.2977	−0.0589	0.9986
245	LAC	0.3435	−0.0165	0.9923
262	LACd	−0.1587	−0.0127	0.9996
283	LACd	−0.1185	0.0263	0.9974
287	LAC	0.0929	0.0184	0.9921

3.2. Multiple linear regression analysis

The same signals used in the above described classical methods can be also used to determine the concentration of each component by MLRA. Such a method allows to calculate a concentration value as a function of several specific signals.

In particular, LACd concentration could be directly carried out through MLRA as a function of $^1D_{301}$ and $^1D_{346–397}$ signals.

$$[\text{LACd}] = 3.1779\ ^1D_{301} - 6.1086\ ^1D_{346–397} - 0.0816$$

$$R^2 = 0.9998$$

Table 4
Accuracy and precision values from replicated assay of prediction sets by all the defined spectrophotometric methods

Analytical technique	Analyte	Signal (nm)	Recovery (%)	RSD (%)
ZCT	LAC	245	98.37	2.16
ZCT	LAC	287	95.11	4.70
ZCT	LACd	220	96.70	8.05
ZCT	LACd	262	91.19	19.83
ZCT	LACd	283	118.91	21.68
Classical	LAC	397–46	98.75	1.53
Classical	LACd	302	91.10	12.68
MLRA	LACd	302; 397-46	96.64	8.49
PCR	LAC	210–340	103.03	3.31
PCR	LACd	210–340	96.27	3.14
PLS	LAC	210–340	101.38	1.42
PLS	LACd	210–340	98.37	3.25

The $^1D_{301}$ signal was due to both components, while the $^1D_{346–397}$ signal allowed to get the LAC concentration. This value was computed in MLRA to subtract the LAC contribution from the $^1D_{301}$ common signal. The $^1D_{346–397}$ signal was already utilized for the direct determination of LAC by a linear equation, as described in the preceding paragraph.

Accuracy and precision results, calculated by applying the MLRA procedure to the prediction set mixtures, are reported in Table 4.

3.3. Zero-crossing methods

The ZCT states that the absorbance measured to a specific wavelength in a derivative spectrum exactly corresponds to the concentration of one component in a mixture, when the absorbance curves of the remaining components cross the abscissa axis. In our case, several suitable signals fitted such a requirement. In particular, the spectral line of LACd crossed the *x*-axis at 245 and 287 nm where the absorbance values could therefore reflect the concentration of the drug only. Fig. 2 shows the first-order derivative spectra of a series of binary mixtures with LAC concentration fixed at 9.83 μg/ml and LACd ranging between 0.51 and 10.20 μg/ml. It was evident that these signals are independent of the LACd concentration and just proportional to LAC concentration.

On the contrary, the signals at 220, 262 and 283 nm, accordingly assigned to LACd absorbance, were demonstrated to be independent of LAC concentration, as shown in Fig. 3.

The relative calibration curves, defined through regression analysis, are reported in Table 3. All these relationships were taken into consideration for the analysis of the prediction set samples. Results were collected in terms of accuracy and precision, as % recovery and % relative standard deviation (RSD), respectively. The statistical values achieved by ZCT signals are reported in Table 4.

3.4. Chemometric analysis

In the spectrophotometry field, the greatest benefits of multivariate analysis versus traditional methods are deriving from the simultaneous use of absorbance values along the full spectrum. The chemometric algorithms used were principal component regression (PCR) and partial least squares (PLS). Models developed by such methods were optimized by selecting the spectral regions bearing the majority of useful information and discarding the interfering noise. A careful investigation was accordingly performed in the optimization step and the spectral region with better information was selected between 210 and 340 nm.

The validation of the models was carried out by a full-cross procedure. According to such process, successive calibrations were performed by removing one standard at a time, then predicting the same sample by the calibration so obtained. The standard error of prediction (SEP) was chosen as an optimizing criterion to select the number of PC. SEP represents an assessment of the error in the prediction of unknown mixtures. The number of PC giving the minimal SEP was considered optimal. The PC number was found to be three for LAC and LACd in the PCR model, while in the PLS method it was found to be two for LAC and four for LACd. PCR and PLS models showed satisfactory SEP values, always under 2, for both the components.

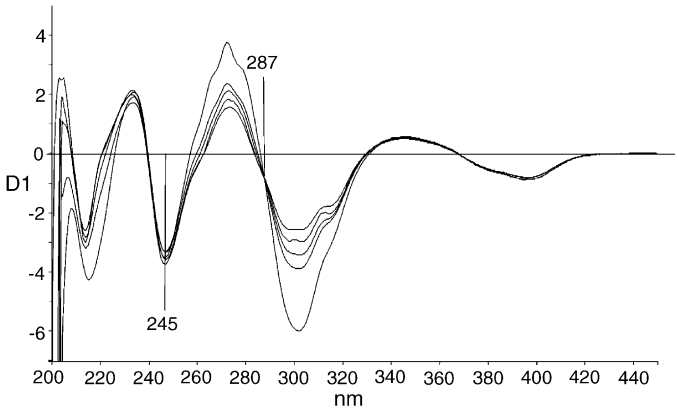

Fig. 2. First-order derivative spectra of binary mixtures with LAC concentration fixed at 9.83 μg/ml and LACd ranging between 0.51 and 10.20 μg/ml.

3.5. Analysis of synthetic and commercial samples

The methods herein developed have been applied to a prediction set built by a full factorial design. The set was composed of 16 synthetic binary mixture with concentration values distributed on four levels. The application of the methods on the prediction set allowed to verify their reliability and predictive power by comparison of accuracy, precision and robustness parameters. The accuracy and precision results have been estimated as % recovery and % RSD for all the methods.

LAC commercial formulations were analogously subjected to analysis by means of the proposed procedures. When applied to pharmaceutics, the methods required only a simple and rapid sample preparation which was demonstrated to avoid any detectable photodegradation during the entire manipulation process.

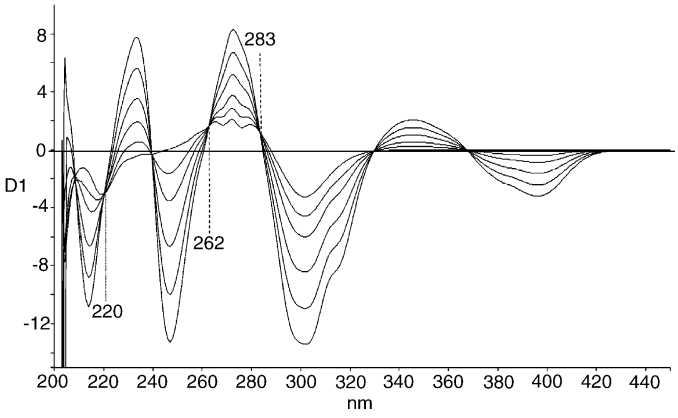

Fig. 3. First-order derivative spectra of binary mixtures with LACd concentration fixed at 10.20 μg/ml and LAC ranging between 4.92 and 39.33 μg/ml.

4. Discussion

The application of the defined methods on the prediction sets showed different prediction power. The recovery of both components, calculated as deviance values from 100, are drawn in Fig. 4. Some methods showed a noteworthy accuracy, while some others furnished a low reliability with deviances up to 20%.

As a first result, a better prediction for LAC with respect to LACd was verified by all the studied methods. This could be easily explained by the clearly higher LAC concentration values adopted either in the calibration set and in the prediction set. The high concentration ratio LAC/LACd was chosen after considering that the photoproduct content in the pharmaceutical formulations resulted very low even after prolonged light exposure. Tablets actually resulted sufficiently stable, with a decrease of LAC title of 8% after 45 h of xenon lamp exposure.

The analysis carried out by ZCT showed heterogeneous results, mostly depending on the nature of the signal used. LAC determination could be performed by two signals at 245 and 287 nm where LACd spectra showed characteristic cross points. The former signal accidentally corresponds to an absorption maximum in the LAC spectrum. In this case, LAC determination resulted very accurate and precise, with a recovery value of 98.37% and RSD of 2.16%. On the contrary, the drug determination carried out by using the signal at 287 nm, placed under the ascending side of a spectral peak, resulted less accurate and precise, with a 95.11% recovery and 4.70% RSD.

Determination by ZCT of the photoproduct LACd might be calculated at three different wavelengths, corresponding to the same cross-points in the LAC spectra. The first signal, centred at 220 nm, corresponded to a maximum peak in the LACd spectrum. On the other hand, the remaining two signals, centred at 262 and 283 nm, corresponded to a descending and ascending sides of the LACd spectral bands, respectively. The use of the first signal allowed LACd determination with reasonable accuracy, equal to 103.70% recovery, but with a low precision of 8.05% RSD. The uncertainty of this determination was supposed to be due to the low stability of the absorbance values associated with the signal considered. In fact, the signals under 220 nm were characterized by higher variance than the rest, depending on a very poor instrumental stability during the spectra recording as well as spectral derivatization.

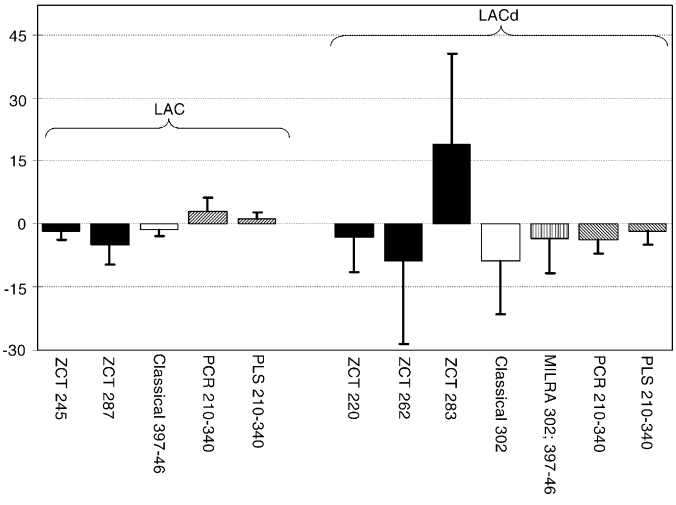

Fig. 4. Recovery, as deviance values from 100, and relative % RSD, calculated from prediction set samples by all the defined methods.

LACd assay by using the other two signals at 262 and 283 nm, placed on slopes of absorption maxima, resulted very less accurate and precise. The replicate assays were characterized by % recovery values of 91.19 and 118.91, whereas the precision values, expressed as % RSD, resulted to be 19.83 and 21.68%, respectively. The low prediction ability of these methods was supposed to be due to the high uncertainty in assessing the absorbance value when a wavelength lying on a slope of a spectral peak was used.

In particular, the poor wavelength precision in ZCT analysis, due to the use of a wavelength lying on a slope of a peak, will result in significant errors in measured absorbance. The wavelength accuracy must be necessarily checked by using narrow transmittance bands achieved by a calibration standard or, more simply, by emission lines of a deuterium lamp, when a zero-crossing procedure is adopted.

Instrumental noise represents another important factor affecting precision and accuracy of the absorbance measurements. In the present study, this factor had to be highly taken into consideration because of the photoproduct concentration, very low if compared with that of the drug. The robustness of the ZCT methods was effectively demonstrated to be directly proportional to LACd concentration The results become unacceptable when LACd concentration fell under a 5%. The poor results carried out in LACd determination by using the signals at 262 and 283 nm were likely due to the assay of samples containing low LACd concentrations.

Conventional spectrophotometric analysis, using a LAC specific peak-through between 397 and 346 nm, always independent of LACd, gave good results for the quantitative analysis of the drug. Recovery ±RSD-values resulted as high as $98.75 \pm 1.53\%$. On the other hand, LACd determination, attempted either by extrapolation of the common 301 nm signal or by MLRA technique, proved to be less satisfactory. Percentage recovery and RSD values, calculated by these methods, resulted to be 91.10 ± 12.68 and 96.64 ± 8.49, respectively. Once again, these results showed that the use of classical spectrophotometric methods, based on the principle of spectrophotometric additivity, often gave unsatisfactory results because of the extensive peaks overlapping. Spectral interferences resulted very critical for LACd, because of the clearly higher concentration of the remaining component.

Chemometric methods worked out by spectrophotometric data proved to have a good analytical reliability when applied to the present mixture. Multivariate calibration methods allow to extract analytical information from the full-spectra of multicomponent samples. However, an optimal selection of the wavelength regions to be used for calibration must be considered in order to improve the models, since not any wavelength would provide useful information. After a deep investigation aimed at optimizing the calculated models, the spectral zone

was restricted to 210–340 nm. The wavelengths below 210 nm were rejected, since characterized by a variance higher than the remaining spectral region, thus furnishing only noisy information to the algorithms. Analogously, the range over 340 nm was discarded, as the absence of LACd absorption in that region furnished a low grade of data variance to the algorithms.

No significant differences in results were obtained when PCR and PLS models were applied to the analysis of the prediction set, thus proving a high resolving power for both algorithms. The concentrations predicted by the models were found to be very close to the nominal concentrations, so demonstrating a high prediction ability for both chemometric methods. The mean % recovery resulted within 101 and 104 with RSD under a 5% for LAC determination, while recovery was in the range 93–97 and RSD under a 6% for LACd.

Chemometric methods gave best results even in the analysis of the pharmaceutical formulations of the drug. The recorded results were in agreement with the content of drug declared on confection labels. The photodegradation product content of assayed pharmaceutical specialties was found anyhow to be lower than 1.1%.

5. Conclusions

A deep statistical study on the use of ZCT in derivative spectrophotometry was performed. The method was compared to other spectrophotometric procedures. The ZCT efficacy was demonstrated to be highly depending on the nature of the signal used. The wavelength precision resulted of paramount importance, since ZCT methods highly resorted to wavelengths lying on slopes of the spectral bands. In such a case, low reliability of ZCT methods was actually demonstrated to be due to the uncertainty in assessing the absorbance value or to low absorbance values. The robustness of the methods was accordingly demonstrated to be a function proportional to analyte concentration.

In conclusion, ZCT seems to be a valid analytical tool, provided that a research grade instrument is utilized. However, the wavelength reliability parameters should be previously assured by an instrumental or external calibration procedure. In this case, statistically significant results can be drawn.

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