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Characterization and analysis of polymorphs by near-infrared spectrometry

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Abstract

The polymorphic purity of drug is of high pharmaceutical interest as it often dictates its bioavailability. In this work, we developed a rapid, efficient method for the characterization and determination of azithromycin polymorphs using near-infrared (NIR) spectrometry. The drug is characterized by comparison with a NIR spectral library that permits one to determine whether the amount of crystalline form contained in an amorphous azithromycin sample exceeds allowed levels. While the crystalline form is a hydrate, the amorphous form is anhydrous; however, the absorption of a small amount of moisture by the drug reduces the spectral differences between the two forms and hinders the establishment of an accurate calibration model. In this work, we determined the crystalline form by using a partial least-squares regression model (PLS1) for calibration and examined the influence of factors such as spectral treatment, wavelength range and moisture content on the results. The high correlation between the spectra for the two forms enabled the development of a PLS2 model for determining both species jointly. The proposed method was validated with a view to its subsequent use in the analytical control of azithromycin.

Keywords: Polymorphism; Azithromycin; Near-infrared spectrometry; Multivariate calibration; Analysis; Partial least-squares regression

1. Introduction

The ability of a substance to occur in two or more different crystalline forms with a differential arrangement and/or conformation of the molecules in the crystal lattice is known as "polymorphism". A large number of drugs exhibit polymorphism and hence differences in physical and chemical properties such as melting point, chemical reactivity, solubility, dissolution rate, optical or electric parameters, vapor pressure or density [1]. These properties have a direct impact on the stability, dissolution rate and bioavailability of the product concerned, which in turn dictate its therapeutic efficacy. Therefore, the characterization of polymorphs is of a high practical interest to the pharmaceutical industry. Polymorphs are usually identified, characterized and determined with structural (X-ray diffraction) [2–4], spectroscopic infra-red (IR), solid-state NMR, Raman) [5] or thermal (differential scanning calorimetry, thermogravimetric analysis) [6] techniques, among others. The process is usually labour-intensive and time-consuming, and hence is scarcely useful for quality control purposes. On the other hand, near-infrared (NIR) spectrometry possesses highly useful features for use in routine control analyses especially prominent among which are its non-invasive nature, the ability to provide measurements and results in a highly expeditious manner, the need for little or no sample preparation and no reagents, the low cost of analyses and the ability to perform simultaneous determinations. In recent years, the number of applications of NIR spectrometry to the identification and determination of polymorphs has grown substantially [7–11].

Azithromycin is the parent compound in a family of antibiotics derived from erythromycin A. It occurs as a crystalline dihydrate ($C_{38}H_{72}N_2O_{12}\cdot 2H_2O$) and in an amorphous anhydrous form ($C_{38}H_{72}N_2O_{12}$).

Most reported methods for the determination of azithromycin have focused on biological samples and liquid chromatography (LC) [12–19]; this technique, however, does not allow the analysis of polymorphic forms. For

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azithromycin to be pharmaceutically useful, it must be present as an appropriate polymorph ensuring efficient absorption by the body and maximization of its effect; also, it should contain no other potentially proprietary polymorphs in amounts exceeding allowed levels as these are considered different products.

In this work, we assessed the use of NIR spectrometry for the expeditious, efficient characterization of the amorphous form of azithromycin and the determination of the crystalline form it may contain.

2. Experimental

2.1. Samples

We used amorphous (A) and crystalline azithromycin samples (C) obtained from various production batches. Both polymorphs were characterized by X-ray diffraction, using a Seifert XDR 3000 T/T diffractometer, a Cu tube ($\lambda = 1.5418$ Å) at 50 kV, 40 mA, an MZVI goniometer equipped with a C3000 microstep controller, a graphite secondary monochromator and a Bragg–Brentano scintillation detector. Diffraction patterns were recorded over the 2θ range from 5° to 25°, using a bandpass of 0.02° and an irradiation time of 10 s in each position.

An overall 90 samples were prepared by weighing various amounts of the two forms (amorphous and crystalline) and blended by hand, in order to obtain a concentration interval of "C" between 0 and 10%, these results are considered as the reference values.

Following recording of their spectra, the mixtures were re-blended and new spectra recorded, the process being repeated until two identical consecutive spectra were obtained.

The moisture content in the laboratory samples was determined with a Karl-Fischer Metrohm 716 DMS Titrino titrator. Because amorphous azithromycin is slightly hygroscopic, moisture analyses were done and NIR spectra recorded on the same day.

2.2. NIR spectrometry

NIR spectra were recorded on a FOSS NIR Systems 6500 spectrophotometer equipped with a rapid-content analyzer (RCA), using the wavelength range of 1100–2500 nm. The instrument was controlled via the software Vision v.2.21.

Spectra were recorded in the reflectance mode; samples were placed in glass cuvettes and turned over with a spatula, and their spectra obtained in triplicate as the result of 32 scans over the range of 1100–2500 nm each. The reference spectrum was obtained from an empty cuvette. The mean for each three spectra was used in the subsequent spectral treatments.

Partial least-squares regression (PLS1) and principal component analysis (PCA) were done as implemented in the software Unscrambler v.7.8. Calibration models were constructed by cross-validation (using the leave-one-out method) and the number of PLS components used was that yielding the minimum in a plot of residual variance versus number of factors.

The best calibration model was taken to be that providing the lowest relative standard error of calibration (% RSEC) and prediction (% RSEP), defined as

$$\text{RSE (\%)} = \sqrt{\frac{\sum_{i=1}^{n}(Y_{\text{pred}} - Y_{\text{ref}})^2}{\sum_{i=1}^{n}(Y_{\text{ref}})^2}} \times 100$$

3. Results and discussion

Fig. 1 shows the XRD patterns for crystalline and amorphous azithromycin; the spectrum is quite well-defined, with sharp lines for the former and no lines for the latter —the broad, smooth bands observed were due to X-ray scattering by the sample. These patterns indicate that two forms are pure, or that the contamination of amorphous form by crystalline and vice versa is lower that its identification limit.

Crystalline azithromycin (a dihydrate) contains a fixed level of moisture (4.6%), whereas amorphous azithromycin is slightly hygroscopic and may contain 1–3% moisture.

The NIR spectrum for the antibiotic reveals that the main spectral differences between the two forms appear in the water absorption region. Fig. 2 shows the NIR spectra for crystalline and amorphous azithromycin samples stored under different moisture conditions (viz. a desiccator, a vacuum stove at 50 °C and a moist atmosphere). The spectra for the samples of amorphous azithromycin containing increasing amounts of moisture exhibit an increase in strength in the band for water, at ca. 1920 nm. No transformation of amorphous to crystalline form is observed during the time of this study.

In order to determine the influence of the presence of variable amounts of moisture on the calibration models used, we prepared mixtures containing variable proportions of crystalline azithromycin that were stored under various moisture conditions. A PCA for these samples (Fig. 3) revealed that the greatest differences arose from the moisture content (first PC) and that the second PC accounted for the variability in the crystalline azithromycin content.

The presence of different amounts of moisture is a major source of variability that entails careful selection of the calibration samples in order to take account of its influence; to this end, we subjected all available samples—containing various amounts of crystalline azithromycin and water—to PCA and selected those on both extremes to construct the calibration set. Thus, the calibration set consisted of 30 samples and the calibration model was constructed by examining various wavelength ranges and spectral treatments (SNV, and first and second derivative). As can be seen from Table 1, the best results (viz. those with the lowest RSE)

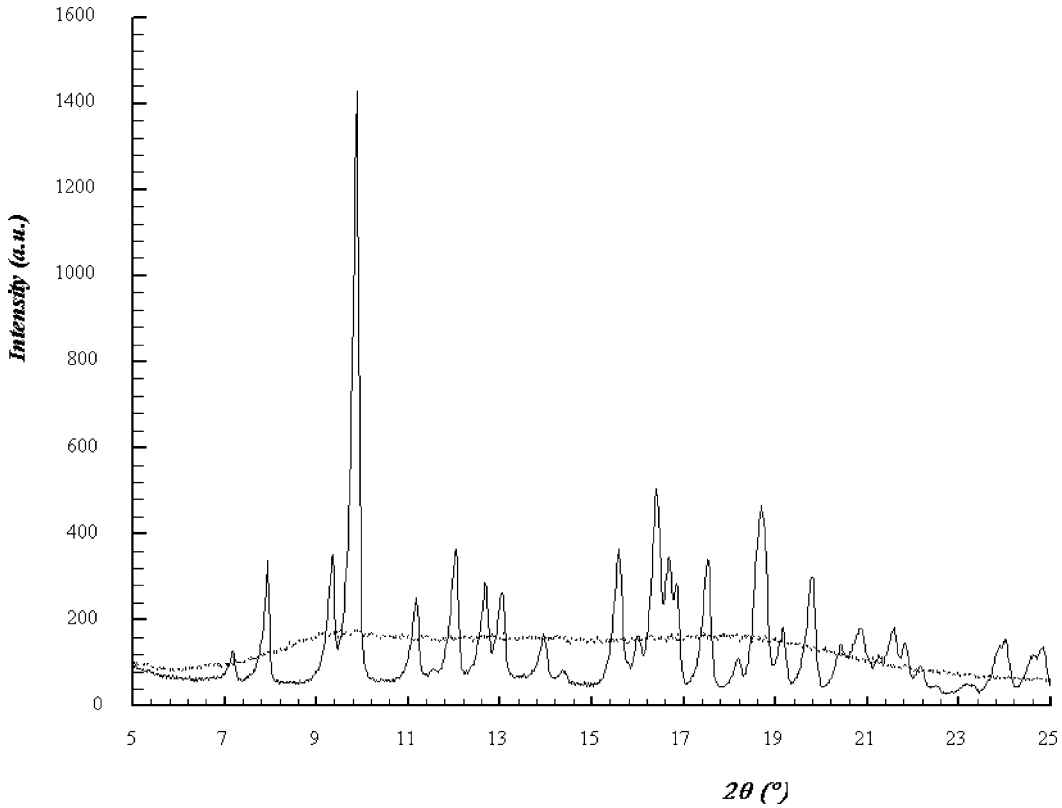

Fig. 1. X-ray diffraction patterns for amorphous and crystalline azithromycin.

were obtained with the second-derivative treatment and the wavelength range of 1800–2200 nm (see Fig. 4), which was the region exhibiting the greatest spectral differences between the two azithromycin polymorphs.

3.1. Determination of moisture

Because absorbed moisture can have a strong impact on the stability of the amorphous form, we developed a method

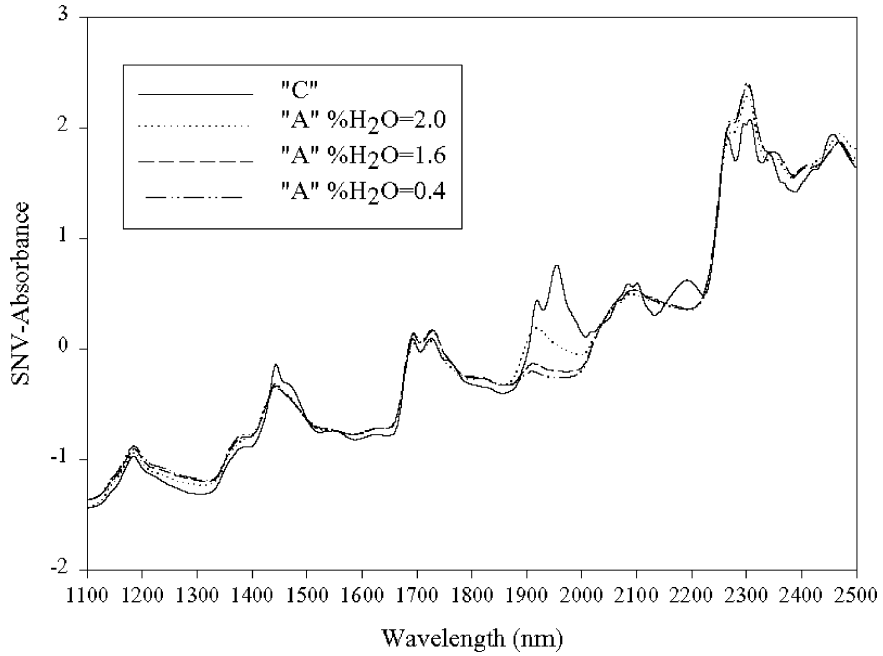

Fig. 2. SNV-corrected absorbance spectra for samples of amorphous and crystalline azithromycin with variable moisture contents.

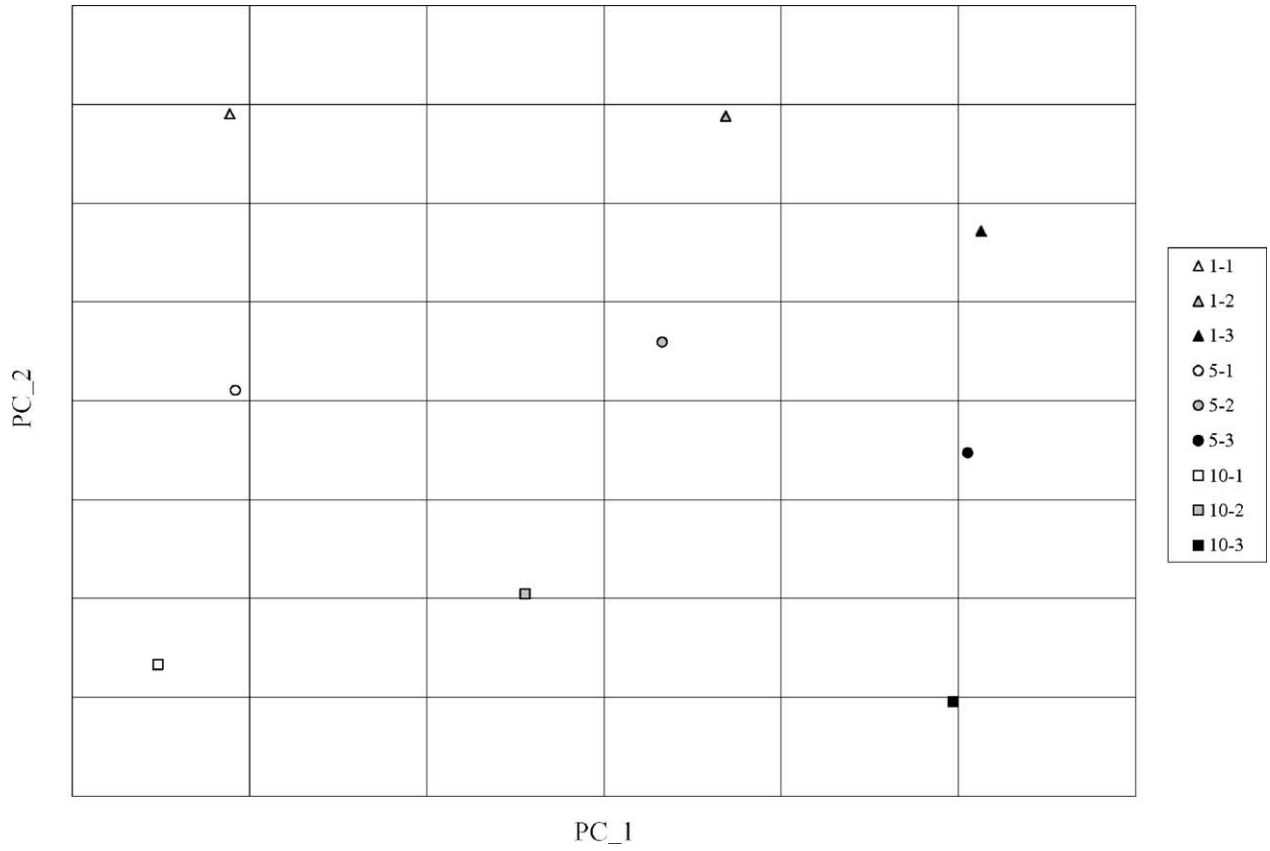

Fig. 3. Scores plot for the first and second principal components for laboratory samples of azithromycin. The first number in the legend denotes the crystalline azithromycin content (%) and the second the moisture content (%).

to quantify it. To this end, we prepared a set of mixtures of both polymorphs that were stored under different environmental conditions in order to obtain variable amounts of moisture, which was quantified by applying the Karl-Fischer method to a series of samples the results for which were used as references in the construction of a NIR spectral model. The calibration set consisted of 17 samples and the prediction set of 25. As in the determination of crystalline

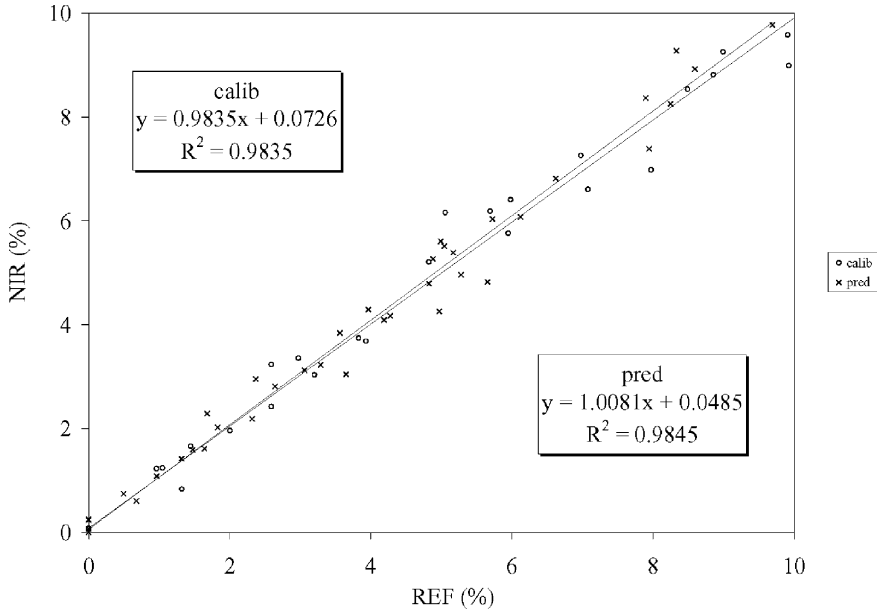

Fig. 4. Plot of NIR predicted vs. reference crystalline azithromycin contents.

Table 1
Relative standard errors for the calibration and prediction sets obtained with the PLS1 models for determining crystalline azithromycin

	Wavelength range					
	1100–2500 nm			1800–2200 nm		
	SNV[a]	1st D[a]	2nd D[a]	SNV[a]	1st D[a]	2nd D[a]
RSEC (%)	37.0	26.1	12.4	38.3	12.4	7.8
RSEP (%)	50.6	38.2	15.5	60.6	14.9	7.9

D: derivative.
[a] Pretreatment.

Table 2
Relative standard errors for the calibration and prediction sets obtained with the PLS1 models for determining moisture

	Wavelength range					
	1100–2500 nm			1800–2200 nm		
	SNV[a]	1st D[a]	2nd D[a]	SNV	1st D[a]	2nd D[a]
RSEC (%)	6.0	3.0	4.0	5.1	3.1	4.0
RSEP (%)	5.9	5.3	5.5	5.8	5.2	5.5

D: derivative.
[a] Pretreatment.

azithromycin, various wavelength ranges and spectral pretreatments (viz. SNV, and first and second derivative) were tested. The results are shown in Table 2. As can be seen, the best results in the two wavelength ranges studied were provided by the first-derivative treatment. Fig. 5 shows the calibration and prediction parameters obtained with the PLS1 model and the first derivative of the variables over the wavelength range of 1800–2200 nm.

The high correlation between the strength of the absorption bands for C and H_2O over the range of 1800–2000 nm led us to use a PLS2 model to determine both analytes jointly (see Table 3). The best model was that using two PCs over the wavelength range of 1800–2200 nm, with the second derivative of the variables. The results for both analytes were accurate and similar to those provided by the PLS1 model for crystalline azithromycin; on the other hand, those for moisture were somewhat higher.

Table 3
Relative standard errors for the calibration (30 samples) and prediction set (42 samples) obtained with the PLS2 models for determining crystalline azithromycin and moisture

	Wavelength range							
	1100–2500 nm				1800–2200 nm			
	1st D[a]		2nd D[a]		1st D[a]		2nd D[a]	
Analyte	C	H_2O	C	H_2O	C	H_2O	C	H_2O
RSEC (%)	26.3	7.5	13.0	8.4	12.4	7.7	7.8	6.2
RSEP (%)	38.4	12.6	16.4	12.4	14.9	12.8	7.9	6.8

D: derivative.
[a] Pretreatment.

3.2. Validation of the proposed method

The proposed method was validated following the ICH Harmonized Tripartite Guideline—Validation of Analytical Procedures: Methodology—[20] according to which the parameters to be evaluated in validating an analytical method such as the one proposed in this work are as follows: selectivity, linearity, range, accuracy, precision (repeatability and intermediate precision), robustness, and limits of detection and quantitation. Determining robustness was judged redundant as the method involved no sample manipulation and the only source of variability pertained to the measuring instrument, which was checked prior to recording every spectrum.

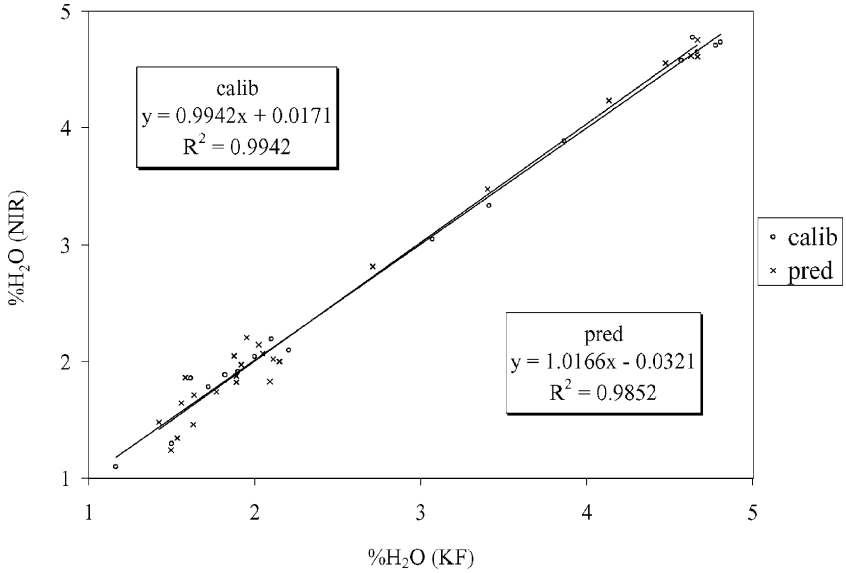

Fig. 5. Plot of NIR predicted vs. reference (KF) moisture contents.

Table 4
Results of the validation of the proposed method for the quantitation of crystalline azithromycin

Parameter	Procedure	Results (PLS1 Model)	
Linearity	[NIR] $= a + b$ [reference]	$n = 9$ Range = 0–10%	$a = -0.09 \pm 0.19$ $b = 1.04 \pm 0.04$ $r = 0.9994$
Accuracy	Paired *t*-test of difference between NIR and reference values	$n = 9$ Average difference = 0.10 S.D. = 0.18 $t_{exp} = 1.662$ $t_{critical} = 2.365$ ($a = 0.05$)	
Repeatability	Three samples in three concentrations level analyzed by the same operator. Calculation of % CV	$x_1 = 0.9$	$CV_1 = 3.1\%$
		$x_2 = 5.1$	$CV_2 = 1.1\%$
		$x_3 = 9.0$	$CV_3 = 2.6\%$
Intermediate precision	Sample analyzed 3 days by two different operators. Calculation of % CV and ANOVA	$x = 5.0$	CV = 3.6%
Detection limit	LD $= 3S.D._a/b$	LD = 0.2%	
Quantitation limit	LQ $= 10S.D._a/b$	LQ = 0.8%	

3.3. Selectivity

We addressed the identification of amorphous azithromycin containing less than 2% of the crystalline form (the maximum allowed level). For this purpose, we constructed a library from spectra for pure amorphous azithromycin and mixtures with proportions below the allowed limit (2%) of the crystalline form. We used the first-derivative spectral mode, the wavelength range of 1800–2200 nm and the residual variance as discriminating parameter, with a threshold of 0.90. We assessed the selectivity of the spectral library thus constructed by using it to identify external samples containing 0–10% crystalline azithromycin. All samples with contents in the crystalline form over the range of 0–2% were positively identified and all with contents outside this range were negatively identified.

3.4. Linearity

This was determined by preparing nine mixtures of amorphous and crystalline azithromycin spanning the calibration range (0–10% of the latter form). Table 4 compares the NIR predicted values (obtained by least-squares regression) with those provided by the reference method. As can be seen, the slope and intercept—given with their respective confidence intervals at a significance level of 95% in the table—were not significantly different from 1 and 0, respectively, so the method can be deemed linear.

3.5. Accuracy

The accuracy of the proposed method was evaluated with the same samples used to examine its linearity. A *t*-test for differences between the proposed and reference methods revealed the absence of significant differences at a confidence level of 95% (see Table 4).

3.6. Repeatability

Repeatability was assessed from the results of triplicate measurements made by the same analyst of samples with three different concentration levels (1.0, 5.0 and 8.9%). As can be seen from Table 4, the coefficients of variation obtained were correct.

3.7. Intermediate precision

This parameter was estimated by having two different workers analyze a sample with a crystalline azithromycin content of 5.0% on three different days (see Table 4). A two-way analysis of variance (ANOVA) of the results revealed the absence of significant between-day or between-analyst differences.

3.8. Limits of detection and quantitation

The limits of detection (LD) and quantitation (LQ) were calculated from the standard deviation of the intercept and slope of the regression graph used in the linearity study (see Table 4). Both were lower than those obtained using X-ray diffraction.

4. Conclusions

It has been confirmed that the present tITP-CE system can be successfully used for direct determination of sub-to-low $\mu g\,l^{-1}$ levels of iodide in seawater. The combination of the proposed tITP conditions and the modified electrolyte composition improves the method's power of detection and performance stability, standing CE to bring it forward for routine analysis purposes. To prove this capability, a systematic monitoring of a more representative set of deep seawater samples (down to 5500 m) is currently underway.

The tITP parameters that can be utilized to determine simultaneously other seawater anionic trace species will also be tested and refined in our forthcoming research.

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