

Quantification of ascorbic acid and sodium ascorbate in powder blends for tableting and in vitamin C chewable tablets by NIR-chemometry

IOAN TOMUȚĂ^{1*}
DANA DUDAȘ¹
ANDREEA LOREDANA VONICA²
SORIN E. LEUCUȚĂ¹

¹ Iuliu Hatieganu University of Medicine and Pharmacy, Department of Pharmaceutical Technology and Biopharmaceutics, 400023 Cluj-Napoca Romania

² S.C. Polipharma Industries, 550052 Sibiu, Romania

The paper proposes a near infrared method able to directly and simultaneously quantify ascorbic acid and sodium ascorbate in powder blends for tableting and in vitamin C chewable tablets without any sample preparation. In the first step, calibration models for the quantification of ascorbic acid and sodium ascorbate in powder blends for tableting and subsequently in chewable vitamin C tablets (corresponding to 80–120 % active substance) were developed according to an experimental design with 2 variables and 5 levels. Then, using the best calibration models, the methods were fully validated in terms of recovery, precision and accuracy for both powder blends and vitamin C chewable tablets. The validated concentration range was 15.14–18.51 % for ascorbic acid and 12.06–14.49 % for sodium ascorbate in powder blends and 91.85–111.03 mg per tablet for ascorbic acid and 71.01–84.50 mg per tablet for sodium ascorbate in tablets. Validation results showed good precision and accuracy.

Keywords: NIR spectroscopy, chemometrics, ascorbic acid, sodium ascorbate, validation, powder blends

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Pharmaceutical controls are time-consuming and require extensive sample preparation. Process analytical technology (PAT) implementation in the pharmaceutical industry would reduce these time consuming operations. To perform this implementation, the use of analytical techniques capable of providing accurate results in a simple and rapid manner is necessary (1). Other advantages include little or no sample preparation (2, 3). Near infrared spectrophotometry (NIR) is a non-destructive and non-invasive technique that allows determination of chemical properties such as active pharmaceutical ingredient (API) or excipient content.

* Correspondence; e-mail: tomutaioan@umfcluj.ro

NIR spectroscopy can be used to perform quantitative determinations of one, two or more compounds in complex matrices like, for instance, a pharmaceutical powder blend for tableting or tablets. A NIR assay method is usually not developed in the »traditional« way as UV spectrometry or HPLC assay methods (4). A major step to develop a NIR assay method is the calibration procedure for model development. Once calibration is developed and favourable predictions are expected, they must be validated to be accepted for routine use. For external validation, independent sets of samples are needed. There are several validation parameters that must be determined in order to be consistent with the recommendations of official guidelines: accuracy, precision (repeatability and intermediate precision), specificity, linearity and range of application (5, 6).

Many papers report the determination of API content in powder blends or tablets by NIR methods (6, 7), but only a few are focused on the prediction of two or more APIs and/or excipients combined in powder blends or tablets (8, 9).

The methods currently used to determine the amount of ascorbic acid and sodium ascorbate in tablets or powder blends for tableting are HPLC (10, 11) and titrimetry (12, 13), but they have the disadvantage of being rather time-consuming. Unlike the conventional methods, NIR-chemometric methods are faster and do not require prior preparation of the sample, so they can be a viable alternative for direct quantification of ascorbic acid and sodium ascorbate in powder blends for tableting and vitamin C tablets. The first objective of our research was to develop a NIR-chemometric method suitable for direct quantification of both APIs from powder blends for tableting.

EXPERIMENTAL

Materials

Ascorbic acid and sodium ascorbate (NPCP, China), sorbitol and mannitol (Roquette, France), orange flavour (Esarom, Romania), sodium saccharin (Niutang Chemical, China), sodium cyclamate (Golden Time Enterprise, China), silicon dioxide – Aerosil (RohmPharma Polymers, Germany) and magnesium stearate (Mosselman, Belgium) were used.

Sample preparation

For calibration purposes, powder blends for vitamin C chewable tablets were prepared. Ascorbic acid, sodium ascorbate, sorbitol, mannitol, sodium saccharin, sodium cyclamate, orange flavour and silicon dioxide were mixed for 5 minutes using a planetary mixer (PRS type, Erweka, Germany) and then passed through a 0.800-mm sieve. Magnesium stearate was then added and mixing was continued for one more minute. The mixture composition was designed for a tablet of a total mass of approximately 600 mg and for 180 mg vitamin C (consisting of 50 % ascorbic acid and 50 % sodium ascorbate in the tablet). This formulation will be further considered as the 100 % active content formulation. Quantitative and qualitative composition of vitamin C chewable tablets are presented in Table I.

Table I. Composition of calibration and validation samples

Content (%)	80 ^a	90 ^{a,b}	100 ^{a,b}	110 ^{a,b}	120 ^a
<i>Vitamin C chewable tablet composition (mg per tablet)</i>					
Ascorbic Acid 97 %	82.4	92.7	103.0	113.3	123.6
Sodium ascorbate	72.0	81.0	90.0	99.0	108.0
Sorbitol	341.8	322.5	303.2	283.9	264.6
Mannitol	90.0	90.0	90.0	90.0	90.0
Orange flavour	1.5	1.5	1.5	1.5	1.5
Sodium saccharin	0.3	0.3	0.3	0.3	0.3
Sodium cyclamate	3.0	3.0	3.0	3.0	3.0
Silicon dioxide	3.0	3.0	3.0	3.0	3.0
Magnesium stearate	6.0	6.0	6.0	6.0	6.0
Tablet mass (mg)	600	600	600	600	600

^a Calibration samples: levels 1-2-3-4-5.

^b Validation samples: levels 2-3-4.

Calibration and validation protocol

The calibration set used included 28 different formulations corresponding to 80, 90, 100, 110 and 120 % ascorbic acid and sodium ascorbate. The samples were prepared according to an experimental design with two factors and 5 levels (Table II) on different days, in the run order. This protocol included batches and days as sources of variability for calibration steps.

To validate the NIR methods for the assay of ascorbic acid and sodium ascorbate, the same formulations as for the calibration set, corresponding to 90, 100 and 110 % ascorbic acid and sodium ascorbate (Table II, samples N7, N13 and N19), were manufactured. At each of the three concentration levels four replicate samples were prepared. In order to include batches and days as sources of variability in validation steps, this protocol was repeated three times on three different days, resulting in a total of 36 validation samples.

NIR spectra recording

Powder blends for tableting were analyzed in a reflection mode using a FT-NIR analyzer (Antaris II, ThermoElectronScientific, USA) equipped with a sample cup spinner for inhomogenous samples, an integrating sphere and an indium gallium arsenide (InGaAs) detector. Reflection spectra were collected with OMNIC software (Thermo Scientific, USA). Each spectrum was the average of 32 scans and the resolution was 8 cm⁻¹ over a range from 11000 to 4000 cm⁻¹. Each powder sample was analyzed three times.

From each sample, fifteen different intact tablets were analyzed in reflection mode using the same FT-NIR analyzer equipped with a universal tablet holder.

Table II. Composition of the calibration set, according to a 2⁵ experimental design

Code	X1	X2	Code	X1	X2
N1	82.4	72.0	N15	123.6	90.0
N2	92.7	72.0	N16	82.4	99.0
N3	103.0	72.0	N17	92.7	99.0
N4	113.3	72.0	N18	103.0	99.0
N5	123.6	72.0	N19 ^c	113.3	99.0
N6	82.4	81.0	N20	123.6	99.0
N7 ^a	92.7	81.0	N21	82.4	108.0
N8	103.0	81.0	N22	92.7	108.0
N9	113.3	81.0	N23	103.8	108.0
N10	123.6	81.0	N24	113.3	108.0
N11	82.4	90.0	N25	123.6	108.0
N12	92.7	90.0	N26	103.0	90.0
N13 ^b	103.0	90.0	N27	103.0	90.0
N14	113.3	90.0	N28	103.0	90.0

X1 – amount of ascorbic acid (mg per tablet); X2 – amount of sodium ascorbate (mg per tablet)

^a Level 1 of validation samples (X1 = 90 %; X2 = 90 %);

^b Level 2 of validation samples (X1 = 100 %; X2 = 100 %);

^c Level 3 of validation samples (X1 = 110 %; X2 = 110 %).

Spectral data analysis

Development of NIR calibration models is an iterative process and consists of checking different spectral pretreatments, as well as their combinations within different spectral ranges. Both the specific spectral regions containing strong bands or the whole spectral range and different spectral pretreatments were tested in order to develop calibration models for ascorbic acid and sodium ascorbate assay in powder blends for tableting and tablets. Development of models was based on the following spectral pretreatments: constant offset elimination (COE), straight line subtraction (SLS), standard normal variate (SNV), minim-maxim normalization (MMN), multiplicative scatter correction (MSC), first-derivative (FD), second-derivative (SD) or combined methods: FD followed by SLS, FD followed by SNV, FD followed by MSC.

The QUANT package of the OPUS 6.5 software package was used for multivariate regressions, with no spectra pretreatment and after applying pre-processing methods. This software allows model validation by full cross-validation and test-validation. For the development of prediction models, the PLS (partial least squares) regression method was used. The models for ascorbic acid and sodium ascorbate assay in powder blends were validated by the full cross-validation method while the models for ascorbic acid and sodium ascorbate assay in tablets were validated by the test-validation method. The model with the highest predictive potential was selected according to conventional criteria: low root mean square error of cross validation (*RMSECV*), low root mean square er-

ror of estimation (*RMSEE*), high coefficient of determination (R^2), small number of PLS factors, and low bias (14).

RESULTS AND DISCUSSION

Ascorbic acid and sodium ascorbate assay in powder blends for tableting: development of models and validation

Twenty eight powder blends containing both ascorbic acid and sodium ascorbate and a NIR-chemometric method to simultaneously and directly quantify the two components was developed and validated.

Model development for API assay consisted of checking different combinations of spectrum pretreatments in association with different selected spectrum regions. PLS regression was performed with the calibration set and cross-validation was carried out for model validation. The first step in model development was to investigate eleven pre-processing methods [models (a)-(k), Table III]. This investigation and the selection of the model with the highest predictive potential were mainly based upon the choice of the number of factors (main components), and calculation of the root mean square error of cross validation (*RMSECV*), root mean square error of estimation (*RMSEE*), bias and R^2 (15). The models studied for ascorbic acid and sodium ascorbate assay in powder blends and their most important characteristics are given in Table III.

The best results obtained after examining different spectral regions were for the models created using the spectral region 10000–4133 cm^{-1} for ascorbic acid and 10000–4480 cm^{-1} for sodium ascorbate. Among the models presented in Table III, it can be seen that for ascorbic acid, model (j) has better prediction ability than other models. The (j) model has higher values for R^2 and lower values for *RMSECV* and bias than other models, notably models (g), (h) and (k). At the same time, it can be seen also in Table III, that for sodium ascorbate, model (h) is the model with a better prediction ability. Based on the mentioned predictive ability criteria (low *RMSEC*, high R^2 , low number of PLS factors and low bias), model (j) was selected as the best fitting model for the ascorbic acid assay and model (h) was selected as the best fitting model for the sodium ascorbate assay.

The predictive ability of the chosen models was checked on independent samples during the method validation step. Accuracy, precision (repeatability and intermediate precision), linearity and specific range of application were the validation parameters. Validation was performed according to the strategy proposed by Hubert *et al.* (16, 17). This approach uses tolerance intervals as statistical methodology, which allows predicting a region of concentrations where each future result has a probability to be defined by the analyst (18). For validation purposes, independent batches comprising 4 replicates of powder blends for tableting at 3 different active levels of APIs (90, 100, and 110 %, *m/m*, formulations N7, N13, N23) were prepared and analyzed on 3 different days, resulting in a total of 36 samples. The accuracy profile has the advantage of taking into account the total error, which is the sum of trueness (systematic error) and precision (random error) (16, 17).

Table III. Type of spectral pre-treatment, R^2 , spectral range selected, number of PLS factors and RMSECV of different models for ascorbic acid and sodium ascorbate assays in powder blends for tableting

Model	(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)
Pre-treatment	None	COE	SLS	SNV	MMN	MSC	FD	SD	FS+ +SLS	FD+ +SVN	FD+ +MSC
Ascorbic acid											
Spectral range selected (cm^{-1})	10000–4133										
Number of PLS factors	10	10	9	9	9	9	10	8	10	10	10
R^2	90.58	91.38	92.61	93.97	92.62	93.92	94.33	95.98	94.61	96.75	95.49
RMSECV	0.687	0.664	0.612	0.559	0.633	0.561	0.540	0.452	0.525	0.396	0.478
Bias (%)	0.011	0.008	0.016	0.010	0.019	0.013	0.004	0.007	0.004	0.004	0.016
Sodium ascorbate											
Spectral range selected (cm^{-1})	10000–4480										
Number of PLS factors	10	9	10	10	9	10	10	10	10	10	10
R^2	91.29	91.7	94.18	94.62	94.69	94.21	97.13	98.19	97.65	97.81	97.34
RMSECV	0.528	0.516	0.428	0.408	0.407	0.427	0.300	0.242	0.268	0.262	0.290
Bias (%)	0.017	0.007	0.003	0.005	0.021	0.004	0.018	0.009	0.017	0.017	0.014

No spectral pre-treatment.

COE – constant offset elimination, SLS – straight line subtraction, SNV – standard normal variate, MMN – minim-maxim normalization, MSC – multiplicative scatter correction, FD – first derivative, SD – second derivative, FS+SLS – first derivative followed by straight line subtraction, FD+SVN – first derivative followed by standard normal variate, FD+MSC – first derivative followed by multiplicative scatter correction.

The validation criteria of the methods developed for ascorbic acid and sodium ascorbate quantitative analysis in powder blends for tableting are given in Table IV. The trueness of the method was evaluated by calculating recovery. The values obtained for recovery were satisfactory for both APIs at all three concentration levels tested, all values being included in the range of 98.8 to 101.7 % (Table IV). The precision of the methods was evaluated by calculating repeatability at the three concentration levels (Table IV). For ascorbic acid, the RSD repeatability ranged from 1.5 to 2.0 %, and for sodium ascorbate, it was 1.2–1.9 %.

Fig. 1a shows the accuracy profile for the ascorbic acid assay in powder blends for tableting, which is based on the validation results obtained with the developed NIR (j) model, FD followed by SNV spectra pretreatments, in the spectral region 10000–4133 cm^{-1} . Similarly, the accuracy profile for the sodium ascorbate assay in powder blends for tableting was built using validation results obtained with the developed NIR (h) model, SD spectra pretreatments, in the spectral region 10000–4480 cm^{-1} (Fig. 1b). As shown in Fig. 1a and in Table IV, accuracy of the method for ascorbic acid in powder blends is good, as the β -expectation tolerance limits do not exceed the ± 5 % acceptance limits. The largest relative tolerance limits were ± 5.0 %, for the lowest ascorbic acid content of pow-

Table IV. Validation results of NIR methods for ascorbic acid and sodium ascorbate assays in powder blends for tableting

Mean API (%)	Recovery (%)	Repeatability (RSD, %)	Accuracy	
			Relative tolerance limits (%)	Tolerance limits (%)
Ascorbic acid				
15.14	100.0	2.0	[-5.0, 5.0]	[14.4, 15.9]
16.83	99.5	1.5	[-4.7, 3.8]	[16.0, 17.5]
18.51	101.7	1.6	[-3.3, 4.7]	[17.9, 19.4]
Sodium ascorbate				
12.06	100.5	1.9	[-4.6, 5.6]	[11.5, 12.7]
13.30	99.7	1.7	[-5.0, 4.4]	[12.7, 13.9]
14.49	98.8	1.2	[-5.0, 2.6]	[13.9, 15.0]

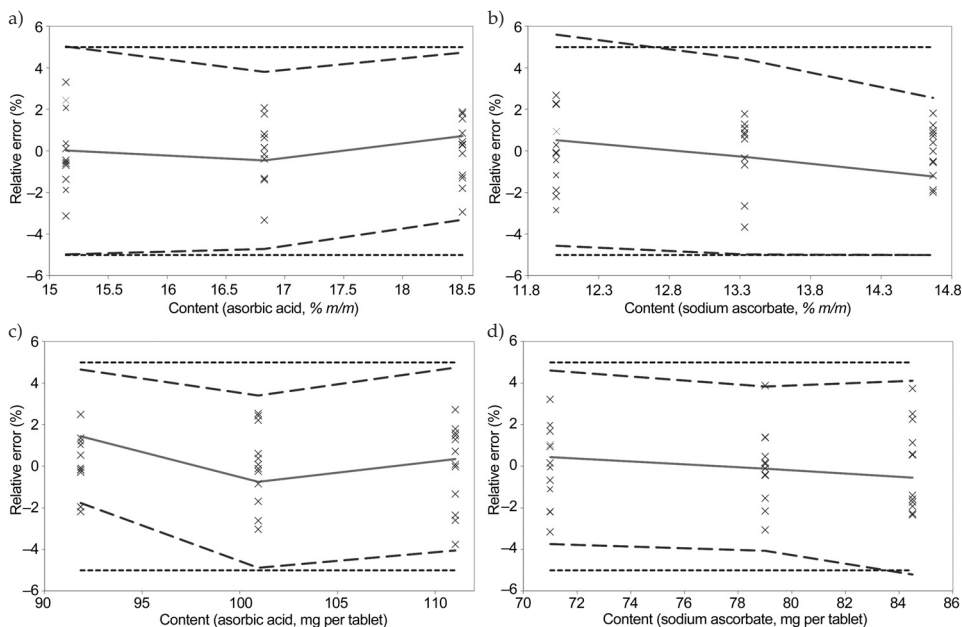


Fig. 1. Accuracy profile of the NIR methods for: a) ascorbic acid, b) sodium ascorbate assay in powder blend for tableting, c) ascorbic acid and d) sodium ascorbate assay in tablets. The full line is relative bias, dashed lines are β -expectation tolerance limits ($\beta = 95\%$) and dotted curves are acceptance limits ($\pm 5\%$).

der blends in validation batches. Similarly, the accuracy of the method for sodium ascorbate in powder blends seems quite good; the β -expectation tolerance limits exceeded only slightly the $\pm 5\%$ acceptance limits (Fig. 1b and Table IV). The largest relative tolerance limits were -5.0% for the highest sodium ascorbate content and 5.6% , for the lowest sodium ascorbate content of powder blends in validation batches. Accuracy profiles included in the $\pm 5\%$ limits give enough confidence for the quality and reliability of the results obtained by the developed NIR-chemometric methods (19).

The linear profile of the prediction models is shown in Figs. 2a and b. The linear model was obtained by plotting calculated concentrations of the validation samples as a function of introduced concentrations. Dashed limits correspond to the accuracy profile and dotted curves represent the acceptance limits at $\pm 5\%$ expressed in concentration units. The linearity profile is within or close to the acceptance limits, the R^2 value is higher than 0.95 and the slope is very close to 1, confirming the linearity of the models for ascorbic acid and sodium ascorbate assays in powder blends for tableting.

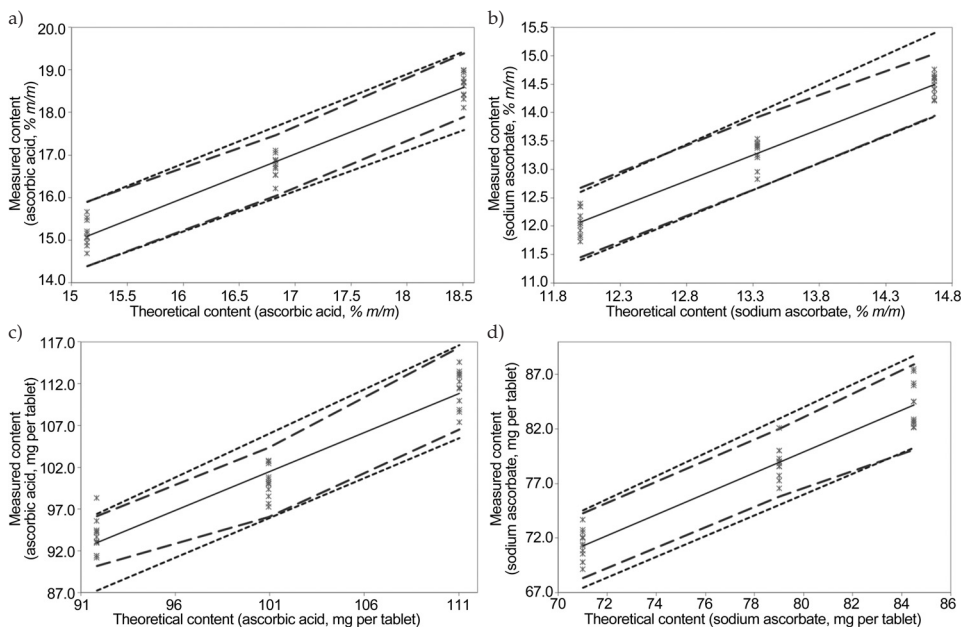


Fig. 2. Linear profile of the NIR methods for: a) ascorbic acid, b) sodium ascorbate assay in powder blend for tableting, c) ascorbic acid and d) sodium ascorbate assays in tablets. Dashed limits correspond to the accuracy profile and dotted curves represent acceptance limits at $\pm 5\%$. Full line is the identity line, $y = x$.

Ascorbic acid and sodium ascorbate assay in vitamin C chewable tablets: development of models and validation

The other aim of our research was to develop a NIR-chemometric method suitable for direct quantification of ascorbic acid and sodium ascorbate in tablets. For this purpose, a protocol consisting of preparing and analyzing 28 of tablet formulations was followed. Practically, the powder blends used for the development and validation of NIR chemometric methods in powder blends for tableting were compressed into vitamin C chewable tablets with a total mass of 600 mg per tablet. The same protocol and methodology as previously described for the development and validation of NIR chemometric methods to simultaneously and directly quantify ascorbic acid and sodium ascorbate in powder blends for tableting was followed for tablets.

Table V shows the main characteristics of the investigated models for ascorbic acid and sodium ascorbate assays in tablets.

Based on the analysis of different spectral regions, the models generated with the use of spectral region 8850–4070 cm^{-1} for both ascorbic acid and sodium ascorbate showed the best results. It can be seen from Table V that for ascorbic acid (j) model is better than other models in terms of prediction. The (j) model has higher R^2 values and lower

Table V. Type of spectral pre-treatment, R^2 , spectral range selected, number of PLS factors, RMSEP of different models for ascorbic acid and sodium ascorbate assays in tablets

Model	(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)
Pre-treatment	None	COE	SLS	SNV	MMN	MSC	FD	SD	FS+ +SLS	FD+ +SVN	FD+ +MSC
Ascorbic acid											
Spectral range selected (cm^{-1})	8850–4070										
Number of PLS factors	8	8	8	7	8	8	10	9	10	10	10
R^2	82.75	84.08	85.31	84.49	86.55	86.19	92.16	85.06	91.81	95.31	94.95
RMSEP	7.10	6.92	6.76	6.87	6.72	6.64	5.74	6.79	5.80	5.21	5.28
Bias (%)	-0.541	-0.551	-0.478	-0.631	-1.22	-1.12	-0.14	0.24	-0.109	-0.097	-0.15
Sodium ascorbate											
Spectral range selected (cm^{-1})	8850–4070										
Number of PLS factors	9	10	10	9	9	9	9	8	9	8	8
R^2	87.50	95.37	95.49	93.71	93.85	93.68	95.69	93.50	96.18	95.14	94.87
RMSEP	3.87	3.40	3.38	3.68	3.66	3.68	3.34	3.71	3.25	3.44	3.49
Bias (%)	0.127	-2.68	-0.201	-0.255	-0.409	-0.27	-0.36	-0.46	-0.262	-0.327	-0.386

No spectral pre-treatment.

COE – constant offset elimination, SLS – straight line subtraction, SNV – standard normal variate, MMN – min-maxim normalization, MSC – multiplicative scatter correction, FD – first derivative, SD – second derivative, FS+SLS – first derivative followed by straight line subtraction, FD+SVN – first derivative followed by standard normal variate, FD+MSC – first derivative followed by multiplicative scatter correction.

values for *RMSEP* and bias than other models, especially (i) and (k) models. Regarding sodium ascorbate, the (i) models prediction ability is better than any one of other models presented in Table V. On these grounds, the best fitting model chosen for the ascorbic acid assay in tablets was model (j) with FD followed by SNV pre-treatment and model (i) with FD followed by SLS pre-treatment was chosen as the most suitable model for the sodium ascorbate assay in tablets.

Table VI shows validation criteria of the developed methods for ascorbic acid and sodium ascorbate assays in tablets. Recovery had satisfactory values at all three concentration levels for both APIs, the lowest value being 98.9 % and the highest 101.5 % (Table VI). The precision of the methods was evaluated by calculating repeatability at the three concentration levels used. Repeatability was good for all studied samples, parameters had satisfactory values at all concentration levels for both APIs, ascorbic acid and sodium ascorbate (Table VI). RSD ranged from 1.6–1.9 % for ascorbic acid and 1.7–2.1 % for Na-ascorbate.

Accuracy profiles for ascorbic acid and sodium ascorbate assays in tablets are given in Figs. 1c and d, respectively. The accuracy profile for the ascorbic acid assay is based on the validation results obtained with the NIR (j) model, FD followed by SNV spectra pretreatments, in the spectral region 8850–4070 cm^{-1} , while the accuracy profile for the sodium ascorbate assay is based on the validation results obtained with the NIR (i) model, with FD followed by SLS spectra pretreatments, in the same spectral region. Since β -expectation tolerance limits are maintained within ± 5 % acceptance limits, the accuracy of the method for the ascorbic acid assay in tablets is considered good (Fig. 1c and Table VI). The largest relative tolerance limits were -4.9 % for the medium ascorbic acid content and 4.8 % for the highest ascorbic acid content of tablets in validation batches. Similarly to the accuracy of the method for sodium ascorbate assay tablets is rather good, the ± 5 % (19) acceptance limits being only slightly exceeded (Fig. 1d) and Table VI). The largest relative tolerance limits were -5.2 % for the highest sodium ascorbate

Table VI. Validation results of NIR methods for ascorbic acid and sodium ascorbate assays in tablets

Mean API content (mg per tablet)	Recovery (%)	Repeatability (RSD, %)	Accuracy	
			Relative tolerance limits (%)	Tolerance limits (mg per tablet)
Ascorbic acid				
91.85	101.5	1.6	[-1.8, 4.7]	[90.2, 96.2]
100.94	99.3	1.8	[-4.9, 3.4]	[96.0, 104.4]
111.03	100.4	1.9	[-4.0, 4.8]	[106.5, 116.3]
Sodium ascorbate ^a				
71.01	100.4	1.8	[-3.7, 4.6]	[68.3, 74.3]
79.01	98.9	1.7	[-4.1, 3.8]	[75.8, 82.0]
84.50	99.5	2.1	[-5.2, 4.1]	[80.1, 87.9]

^a Expressed on ascorbic acid equivalence basis.

content and 4.6 % for the lowest sodium ascorbate content of tablets in validation batches.

Figs. 2c and d represent linear profiles of the prediction models. Again, the linearity profile is within the acceptance limits, the R^2 value is higher than 0.95 and the slope is very close to 1. This confirming the linearity of the models for ascorbic acid and sodium ascorbate assays in tablets.

CONCLUSIONS

According to the data presented, the NIR-chemometric methods using the (j) model for ascorbic acid and the (i) model for sodium ascorbate have good reproducibility and satisfactory accuracy and linearity profiles. After analyzing the statistical parameters, it can be concluded that both NIR-chemometric methods are linear and sufficiently precise and accurate for simultaneous and direct quantification of the two components (ascorbic acid and sodium ascorbate) in powder blends for tableting, and in chewable tablets.

In conclusion, the overall results of this paper indicate that near infrared spectroscopy methods are appropriate and advantageous for directly predicting, without any sample preparation, of ascorbic acid and sodium ascorbate in powder blends for tableting and in vitamin C chewable tablets. Such rapid NIR-chemometric methods can be used for on-line, in-line or at-line monitoring of the manufacturing process of vitamin C chewable tablets and are helpful in achieving the goals of the process analytical technology concept.

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