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Determination of sugars in depilatory formulations: A green analytical method employing infrared detection and partial least squares regression

Mercedes Cascant, Julia Kuligowski, Salvador Garrigues, Miguel de la Guardia*

Department of Analytical Chemistry, University of Valencia, Edificio Jerónimo Muñoz, 50th Dr. Moliner, 46100 Burjassot, Spain

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ABSTRACT

A green analytical method was developed for the analysis of sugar-based depilatories. Three independent partial least squares (PLS) regression models were built for the direct determination of glucose, fructose and maltose without any sample pretreatment based on their attenuated total reflectance – Fourier transform infrared (ATR-FTIR) spectra. The models showed adequate prediction capabilities with root-mean-square-errors of prediction ranging from 7.04 to 12.55 mg sugar g^{-1} sample. As a reference procedure, gradient liquid chromatography with on-line infrared detection, employing background correction based on cubic smoothing splines, was used. The analysis revealed changes in the sugar concentration due to the formulation process as compared to information on the ingredients provided by the manufacturers. Although fructose, glucose and sucrose were declared to be used for the production of depilatories, in the final products only fructose, glucose and maltose were determined. This fact was attributed to pH and temperature conditions employed during the production process as well as to the use of glucose syrup instead of crystalline glucose. The present ATR-FTIR-PLS method enables an accurate, cheap and fast determination without solvent consumption or toxic waste generation and offers therefore a green screening alternative to methods employing chromatographic techniques.

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1. Introduction

Among the different depilatory products available on the market, a group of depilatories elaborated on the basis of sugars can be identified. As raw materials mainly sucrose, glucose, fructose and honey are employed in such products, together with other minor compounds like glycerin, water, citric acid and fragrances. During the production process, the raw materials are blended at elevated temperatures according to the specific formulations of each product which can modify the nature and concentration of sugars. However, to the best of our knowledge, no references concerning the analysis of the sugar composition of depilatories can be found in the literature.

For the determination of sugars, liquid chromatography (LC) followed by refractive index (RI) detection is frequently employed. However, this detection system is characterized by its poor sensitivity, high instability with regard to fluctuations in the mobile phase composition and temperature, low selectivity and incompatibility with gradient conditions [1,2]. Evaporative light scattering detectors are a commonly used alternative. This detector is compatible with gradient elution and shows a significantly higher sensitivity than RI detectors, but its selectivity is also poor [3]. Due to the low absorption coefficients of sugars in the ultraviolet (UV) region, UV detection is only feasible employing pre- or post-column derivatization or using short wavelengths which increases the number of possible interferents, thus requiring an extensive sample clean-up prior to the analysis [1,4]. Mass spectrometry is a very sensitive, selective and versatile detector and its application is therefore focused on the detection of analytes at trace levels. Its use is not recommendable for the analysis of major compounds as it is the case dealing with the determination of sugars in depilatories.

Alternatively, infrared (IR) spectrometry showed its potential as a detector in on-line and off-line hyphenated systems in combination with different separation techniques [5,6]. An important milestone which has contributed to increase the applicability of this detection system was the development of chemometric background correction methods to compensate changing mobile phase contributions to the overall signal and thus, making the measurement under gradient conditions feasible [7].

At the same time, in the last decade, methods based on the direct determination of analytes using attenuated total reflectance (ATR) infrared spectroscopy in combination with multivariate chemometric techniques have been emerging as alternative direct methodologies for the analysis of complex samples and an increasing number of publications dealing with the quantification of multiple analytes can be found [8–11]. The objective of these so-called "green analytical methods" is to replace traditional time

* Corresponding author. Tel.: +34 96 354 4838; fax: +34 96 354 4845.
E-mail address: miguel.delaguardia@uv.es (M. de la Guardia).

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consuming chromatographic procedures, involving the use of solvents and generating toxic residues, by reagent free methods [12].

The objective of the present study was the development of a quantitative and direct method for the determination of sugars in depilatories which could be easily implemented by manufacturers for quality control, avoiding the consumption of solvents during analysis and sample preparation. In a first step, a recently developed on-line gradient LC-IR method for the determination of fructose, glucose, sucrose and maltose in beverages [13] was adapted for the analysis of a series of commercially available depilatories, using a method based on cubic smoothing splines (CSS) [7] for background correction. Afterwards, the obtained sugar concentrations were used as reference values to calculate partial least squares (PLS) regression models for the direct determination of the considered sugars employing ATR-Fourier transform infrared (FTIR) spectra of the samples.

2. Materials and methods

2.1. Standards and samples

D(−)-Fructose, anhydrous D(+)-glucose and maltose-1-hydrate from Panreac (Barcelona, Spain) and D(+)-sucrose from Scharlau (Barcelona, Spain) were used as standards. Twelve depilatory samples were obtained from the local market and acetonitrile (HPLC grade) was purchased from Scharlau (Barcelona, Spain). High-purity water, with a resistivity higher than 18.2 MΩ, was obtained from a Milli-Q water-purification system (Bedford, MA, USA).

2.2. LC-IR reference procedure

For the chromatographic analysis of depilatory samples a recently published LC-IR method for the determination of sugars was adapted [13]. The chromatographic separation was carried out using a Dionex (Sunnyvale, CA, USA) P680 high performance liquid chromatography system, equipped with a Kromasil 100 NH_2 column (250 mm × 2 mm, 5 μm) and a sample injection loop of 20 μl. Linear acetonitrile:water gradients were run from 75% to 55% (v/v) acetonitrile in 15 min.

On-line hyphenation to the FTIR spectrometer was carried out employing a standard micro flow cell with CaF_2 and ZnSe windows and a pathlength of 10 μm. The cell was installed on a Bruker (Bremen, Germany) IFS 66/v FTIR spectrometer equipped with a liquid nitrogen refrigerated mercury–cadmium–telluride (MCT) detector, a vacuum system and a dry air purged sample compartment for FTIR spectra acquisition. The scanner for the interferometer was operated at a HeNe laser modulation frequency of 100 kHz. Spectra were acquired in the range between 4000 and 950 cm^{-1} with a resolution of 8 cm^{-1} and employing a zero filling factor of 2. As a background, the spectrum of the empty sample compartment was recorded. Accumulating 25 scans per spectrum, a spectra acquisition frequency of 15 spectra min^{-1} was provided. Solvent gradient background correction was carried out using a previously developed method based on the use of cubic smoothing splines (CSS) [7] and linear calibration lines were established by measuring the corresponding peak areas. The obtained concentrations of the different sugars in the samples were used as reference values for building the ATR-FTIR-PLS models.

Sugar standard mixtures were prepared by accurately weighing in different amounts of pure sugar standards and dissolving them in 5 ml acetonitrile:water (50:50 v/v) covering a concentration range from 2.5 to 10 mg ml^{-1} for each sugar. For sample preparation, 2.5 ml of water were added to 150–200 mg of each sample. After 25 min of sonication in an ultrasonic water bath (JP Selecta, Barcelona, Spain), all sugars could be completely dissolved and the samples were taken to a final volume of 5 ml with acetonitrile. The solutions were centrifuged at 2500 rpm during 15 min to eliminate un-dissolved particles. All standards and sample solutions were filtered through a 0.22 μm nylon syringe filter prior to their injection into the chromatographic system.

2.3. ATR-FTIR-PLS procedure

For ATR spectra acquisition, a dry-air purged in-compartment DuraSampleIR accessory from Smiths Detection Inc. (Warrington, UK) equipped with a three reflection diamond/ZnSe DuraDisk plate was installed on the spectrometer described in the previous section. Spectra were recorded in the range between 4000 and 600 cm^{-1}, with a spectra resolution of 4 cm^{-1}, averaging 100 scans per spectrum. A spectrum of the clean ATR crystal, obtained immediately before acquiring the spectrum of each sample, was used as a background. Duplicate spectra of 12 depilatory samples were obtained directly by depositing a sample aliquot onto the ATR crystal. Between samples, the crystal was carefully cleaned using distilled water. In addition, 33 mixed samples were prepared by accurately weighing different amounts of the pure samples ranging between 0.4 and 3.1 g. The mixtures were heated during 3 min in a temperature controlled water bath at 80 °C, homogenized by vortexing and placed in an ultrasonic bath for 40 min. ATR spectra were obtained in the same way as described for the pure samples, after cooling down to room temperature. Means of the duplicate spectra of each sample were calculated and employed to build up the PLS models. The obtained data set was divided into a calibration and a validation subset with 26 and 19 objects, respectively. Each subset contained spectra from pure as well as mixed samples. The characteristics of both sets are shown in Table 1. The sugar concentrations of the 12 depilatory samples were determined employing the LC-IR reference procedure and those in the mixed samples were calculated according to the contents of the original samples and the weighed masses. The *y* vector containing the reference sugar concentration values was mean centered to ensure that the criterion for choosing the number of latent variables was only based on the explained variation and not on the absolute concentration.

2.4. Software and algorithms

The chromatographic system was controlled using Chromeleon 6.40 from Dionex (Sunnyvale, CA, USA). For instrumental and measurement control of the IR spectrometer as well as for data acquisition, Opus 6.5 from Bruker (Bremen, Germany) was used. Background correction and data treatment were carried out using in-house written functions employing Matlab 7.7.0 from Mathworks (Natick, MA, USA) and for PLS model calculation and validation the PLS Toolbox 5.8 from Eigenvector Research Inc. (Wenatchee, WA, USA) was used. Detailed information on PLS regression and other related parameters employed in this work have been discussed earlier [14,15].

3. Results and discussion

3.1. Mid-IR spectra of sugars and depilatory samples

In Fig. 1a ATR spectra of fructose, glucose, sucrose and maltose in their solid crystalline form are depicted in the region between 1800 and 600 cm^{-1}, where all four sugars show typical absorption bands. Additionally the compounds show bands in the region around 3000 cm^{-1} due to CH_2 and CH_3 stretching vibrations (data not shown). A detailed band assignment can be found elsewhere [16–18]. In the spectral region between 1500 and 950 cm^{-1}, the four studied compounds show strongly overlapping absorption bands mainly caused by C–O and C–C stretching and C–OH deformation

Table 1
Characteristics of the different data sets used for PLS model calculation and validation.

Set	Type of samples	Number of samples	Mean value (mg g^{-1} sample)			Minimum value (mg g^{-1} sample)			Maximum value (mg g^{-1} sample)			Standard deviation (mg g^{-1} sample)		
			Fructose	Glucose	Maltose	Fructose	Glucose	Maltose	Fructose	Glucose	Maltose	Fructose	Glucose	Maltose
Calibration	Pure and mixed samples	26[a]	96.1	344.6	58.7	0.0	269.1	0.0	284.7	460.4	223.0	61.6	48.2	53.2
Validation	Pure and mixed samples	19[b]	102.3	347.0	49.3	0.0	250.3	0.0	250.3	433.8	149.2	62.2	50.3	42.5

[a] For the calculation of the PLS model for the determination of glucose, one spectrum of the calibration set was identified as an outlier and had to be eliminated prior to model calculation, reducing the number of samples to 25.
[b] For the validation of the PLS model for the determination of fructose and glucose, one spectrum of the calibration set was identified as an outlier and had to be eliminated prior to model calculation, reducing the number of samples to 18.

Fig. 1. ATR reference spectra of solid sugar standards in their crystalline form (a) and ATR spectra of the 12 investigated samples (b). Note: spectra of sugar standards have been normalized and shifted in the *y* direction to clearly show their differences.

modes. This overlapping impedes the direct determination of each sugar contained in the mixture employing univariate calibration methods. However, differences in band shapes and positions of the maxima between the four investigated sugars can be appreciated. Multivariate calibration methods are able to exploit those small differences to quantify the analytes in the presence of interfering substances with similar spectra, when spectral information on the interferents is included in the data set used for model calibration. Fig. 1b shows ATR spectra of the 12 investigated depilatory samples. The spectra of the different samples are highly similar. Basically, absorbance bands in the same regions as discussed before can be observed, reflecting clearly the presence of a mixture of different sugars in the samples.

3.2. Determination of sugars using the LC-IR reference procedure

Table 2 lists the sugars employed in the fabrication of the depilatories evaluated throughout this study as provided by the manufacturers. It should be highlighted, that according to this information sucrose was used in the production of all samples, whereas to none of the sample maltose was added. Additionally, fructose, glucose or honey were added to some samples. Honey

Table 2
List of ingredients provided by the sample manufacturers together with the concentrations of fructose, glucose, sucrose and maltose found employing the LC-IR method.

Sample (#)	Ingredients (provided by manufacturers)					Concentrations determined by LC-IR (mg g^{-1} sample)			
	Fructose	Glucose (syrup)/dextrose	Sucrose	Maltose	Honey	Fructose	Glucose	Sucrose	Maltose
1	No	Yes	Yes	No	Yes	64.1	294.1	<LOD	107.1
2	No	No	Yes	No	No	147.1	299.3	<LOD	<LOD
3	No	Yes	Yes	No	No	<LOD	429.0	<LOD	<LOD
4	No	Yes	Yes	No	No	<LOD	433.8	<LOD	<LOD
5	Yes	Yes	Yes	No	Yes	79.4	269.1	<LOD	223.0
6	No	Yes	Yes	No	No	73.9	340.2	<LOD	107.4
7	Yes	Yes	Yes	No	No	110.4	366.7	<LOD	72.5
8	No	No	Yes	No	No	250.3	397.2	<LOD	<LOD
9	No	No	Yes	No	No	284.7	402.2	<LOD	<LOD
10	No	Yes	Yes	No	Yes	113.9	208.2	<LOD	53.0
11	No	No	Yes	No	No	<LOD	460.4	<LOD	<LOD
12	No	Yes	Yes	No	No	89.2	363.8	<LOD	68.2

Note: <LOD means lower than the limit of detection of the chromatographic method which was 10, 12.5, 22.5 and 32.5 mg g^{-1} sample for fructose, glucose, sucrose and maltose, respectively for a sample amount of 200 mg. LODs were established as three times the standard deviation of three independent measurements at a concentration level of 3 mg ml^{-1} divided by the analytical sensitivity.

mainly contains glucose and fructose, followed by maltose and other sugars in minor concentrations [19] and therefore its addition alters the final sugar concentration.

To determine the concentration of glucose, fructose, maltose and sucrose in the depilatory samples, calibration lines were established from the analysis of different standard mixtures of the four investigated sugars using the LC-IR reference method. The obtained data sets were background corrected using the CSS approach to compensate changes in the baseline due to gradient elution. Three different chromatographic traces were extracted for the quantification of fructose, glucose as well as sucrose and maltose, measuring the absorbance at 1069, 1080 or 1065 cm^{-1} using a single point baseline correction at 1204, 1184 or 1177 cm^{-1}, respectively. For each sugar, a calibration line was calculated by means of a linear regression of the chromatographic peak areas vs. the analyte concentration. The obtained results are shown in Table 3. Obtained limits of detection and quantification (LODs and LOQs) as well as the repeatability were suitable for the determination of sugars in depilatory samples. It has to be remarked that for the analysis of real samples, the sample amounts used in the preparation step were adjusted to suit the LODs and LOQs of the LC-IR method. Figures of merit of the method were comparable to those reported in a previous study focused on the analysis of fructose, glucose, sucrose and maltose in non-alcoholic beverages employing on-line gradient LC-IR [13].

Fig. 2 shows background corrected chromatograms obtained during the injection of a standard solution containing fructose, glucose, sucrose and maltose together with those corresponding to the injection of a sample. From the trace obtained from a standard mixture, it can be seen that the four sugar peaks are baseline resolved. The retention times of fructose, glucose, sucrose and maltose were 12.1, 13.5, 16.1 and 17.9 min, respectively. Similar results were observed during the injection of the samples. Slight shifts in retention times were attributed to temperature changes. Fig. 3

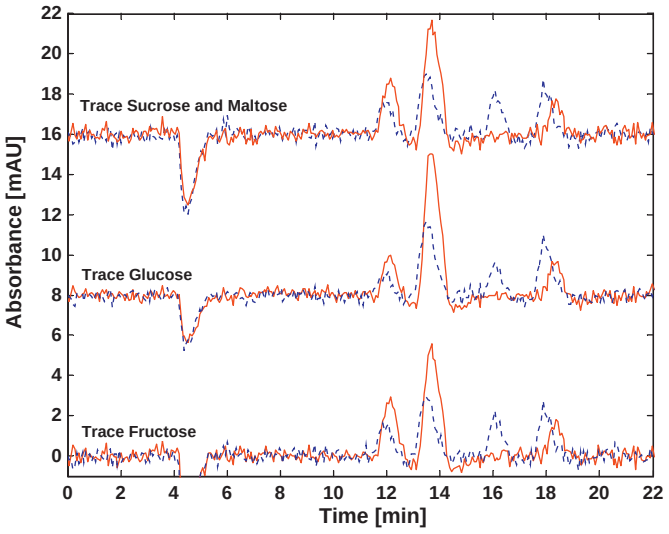

Fig. 2. LC-IR Chromatograms obtained after background correction. Signals were obtained from data acquired during the injection of a standard solution containing 2.66, 7.60, 3.04 and 7.74 mg ml^{-1} of fructose, glucose, sucrose and maltose, respectively (dashed lines) and a sample (continuous lines). Note: traces of fructose, glucose and sucrose and maltose were extracted as described in the text. If necessary, traces have been shifted in the *y* direction.

Table 3
Figures of merit of the LC-IR calibration lines obtained for the four investigated sugars.

Analyte	Concentration range (mg ml^{-1})	$y=(a\pm s_a)+(b\pm s_b)x$[a]		R^2	Noise[b] (mAU)	LOD[c] (mg ml^{-1})	LOQ[d] (mg ml^{-1})	Repeatability[e] (%)
		$(a\pm s_a)$	$(b\pm s_b)$					
Fructose	2.7–10.2	0.0 ± 0.1	0.42 ± 0.02	0.990	0.180	0.4	1.3	2.6
Glucose	2.5–10.3	0.0 ± 0.1	0.32 ± 0.01	0.990	0.154	0.5	1.8	4.2
Sucrose	3.0–10.5	0.1 ± 0.1	0.40 ± 0.03	0.96	0.181	0.9	3.2	5.2
Maltose	2.6–10.0	0.1 ± 0.1	0.25 ± 0.01	0.98	0.181	1.3	4.3	3.2

[a] Calibration curve from 6 standard solutions; *a* and *b* are the intercept and the slope of the calibration lines.
[b] Chromatographic noise measured as the root mean square (RMS) of the chromatographic signal extracted from a background correct injection of a standard mixture between 6 and 10 min, where no analyte signals were present.
[c] Limit of detection established as three times the standard deviation of three independent measurements at a concentration level of 3 mg ml^{-1} divided by the analytical sensitivity.
[d] Limit of quantification established as ten times the standard deviation of three independent measurements at a concentration level of 3 mg ml^{-1} divided by the analytical sensitivity.
[e] Relative standard deviation for three independent measurements carried out at a concentration level of 3 mg ml^{-1}.

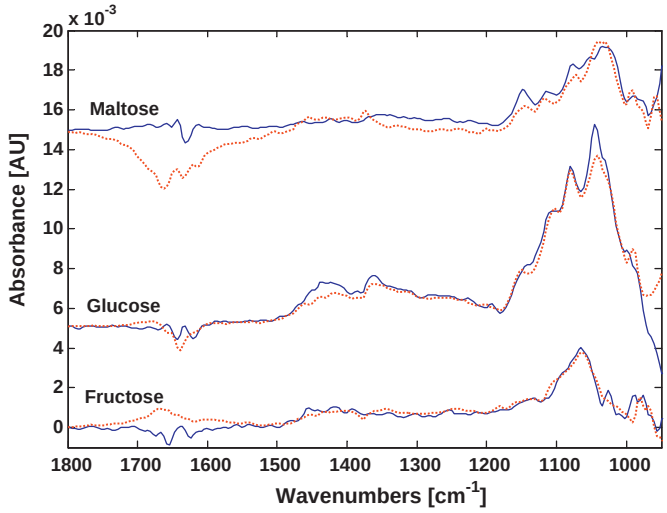

Fig. 3. Recovered sugar spectra extracted from the peak apex after background correction (dashed lines) obtained from the injection of a sample into the LC-IR system together with the recovered sugar spectra obtained from the injection of a standard solution containing 2.66, 7.60, 3.04 and 7.74 mg ml^{-1} of fructose, glucose, sucrose and maltose, respectively (continuous lines). Note: if necessary, spectra were scaled to facilitate comparison; glucose and maltose spectra were shifted in the *y* direction.

shows spectra extracted from the peak apex of each analyte during the injection of a standard mixture in comparison to those obtained during the injection of a sample. As in none of the samples sucrose could be detected, this analyte was not shown in Fig. 3. The analytes present in the samples could be identified comparing the obtained background corrected spectra to those obtained from the standard mixture, even when minor shifts in retention time were observed. For the depicted spectra, correlation coefficients of 97.5%, 99.3% and 96.2% were obtained measuring the absorbance between 1500 and 1000 cm^{-1}, thus confirming the identity of each analyte being all correlation coefficients equal or higher than 95% [20].

Table 2 shows the concentrations of sugars in the depilatory samples using the calibration lines indicated in Table 3. It is remarkable that, although according to the manufacturer's information sucrose was added to all samples during the fabrication process, in none of the samples, sucrose was detected. As sucrose is a disaccharide composed by one unit of fructose and one unit of glucose, a possible explanation for the absence of sucrose in depilatory samples is that it is degraded into fructose and glucose due to pH and temperature conditions employed during the production process [21]. On the other hand, it should be emphasized that in 50% of the samples maltose was found, although apparently this sugar was not added to any sample. This could be explained by the frequent use of glucose syrup, instead of crystalline glucose, in the production of cosmetics. Glucose syrup is obtained from the enzymatic cleavage of starch, a long chain of glucose molecules, and usually glucose disaccharides (maltose) remain in the glucose syrup [22].

Comparing the similarities and differences between the twelve investigated samples, they can be divided into three groups. Most of the samples (samples 1, 3, 4, 6, 10 and 12) were produced using glucose and sucrose. Taking into account that the employed glucose possibly was glucose syrup and that the sucrose degraded to fructose and glucose, it can be explained that in samples 1, 6, 10 and 12, fructose, glucose and maltose were detected. In samples 3 and 4 only glucose was detected which might be due to the negligible amount of sucrose added to the samples. Considering the aforementioned samples sucrose was only found in the third place on the ingredients list. To the second group of samples (samples 2, 8, 9 and 11) only sucrose was added during the production process and in all the cases glucose and fructose were detected using the LC-IR method. The only exception was sample 11, where again only glucose was found. In this case, a possible explanation is that the information on the ingredient list was inaccurate. The last group of samples was elaborated on the basis of fructose, glucose and sucrose (samples 5 and 7) and in both cases fructose, glucose and maltose were detected, as expected.

3.3. Development of a direct ATR-FTIR-PLS procedure for the determination of sugars

As aforementioned, results obtained from the LC-IR analysis of the samples were used as reference values for the calculation of multivariate models for the direct determination of the sugar content in samples using ATR-FTIR spectra. An appropriate variable selection prior to the PLS model calculation provides significant improvements in model performance [23] reducing both, model complexity and the likelihood of model overfitting. As the analytes of interest show bands in the carbohydrate region around 3000 cm^{-1} as well as in the fingerprint region, the independent use of both regions and their combination was investigated. From results obtained (data not shown), the spectral region between 1794 and 600 cm^{-1} was selected, because the inclusion of additional spectral regions did not improve the predictive capabilities of the multivariate PLS models.

Different spectral pre-processing strategies, including the use of 1st and 2nd derivative spectra, mean centering and autoscaling as well as their combinations were also tested. It was concluded that the best results were obtained by the use of the 1st or 2nd derivative row vectors resulting from a 9 point cubic Savitzky–Golay function followed by mean centering (see Table 4). For model evaluation,

Table 4
Summary of the most important calibration and prediction parameters of the ATR-PLS models developed for the determination of fructose, glucose and maltose in depilatories. Concentrations are expressed in mg g^{-1} sample.

Analyte	Pre-processing	LVs[a]	RMSEC[b]	RMSECV[c] (mg g^{-1} sample)	RMSEP[d] (mg g^{-1} sample)	CV[e] bias (mg g^{-1} sample)	Pred[f] bias (mg g^{-1} sample)	R^2 Cal[g]	R^2 CV[e]	R^2 Pred[f]
Fructose	2nd derivative, mean centering	3	6.48	7.17	7.04	−0.36	−3.97	0.990	0.990	0.992
Glucose	1st derivative, mean centering	5	6.76	12.27	12.55	−1.78	2.78	0.98	0.93	0.94
Maltose	1st derivative, mean centering	6	6.27	27.53	7.45	2.79	−3.23	0.990	0.76	0.990

[a] LVs stands for latent variables.
[b] RMSEC stands for root mean square error of calibration.
[c] RMSECV stands for root mean square error of cross validation.
[d] RMSEP stands for root mean square error of prediction.
[e] CV stands for cross validation.
[f] Pred stands for prediction.
[g] Cal stands for calibration.

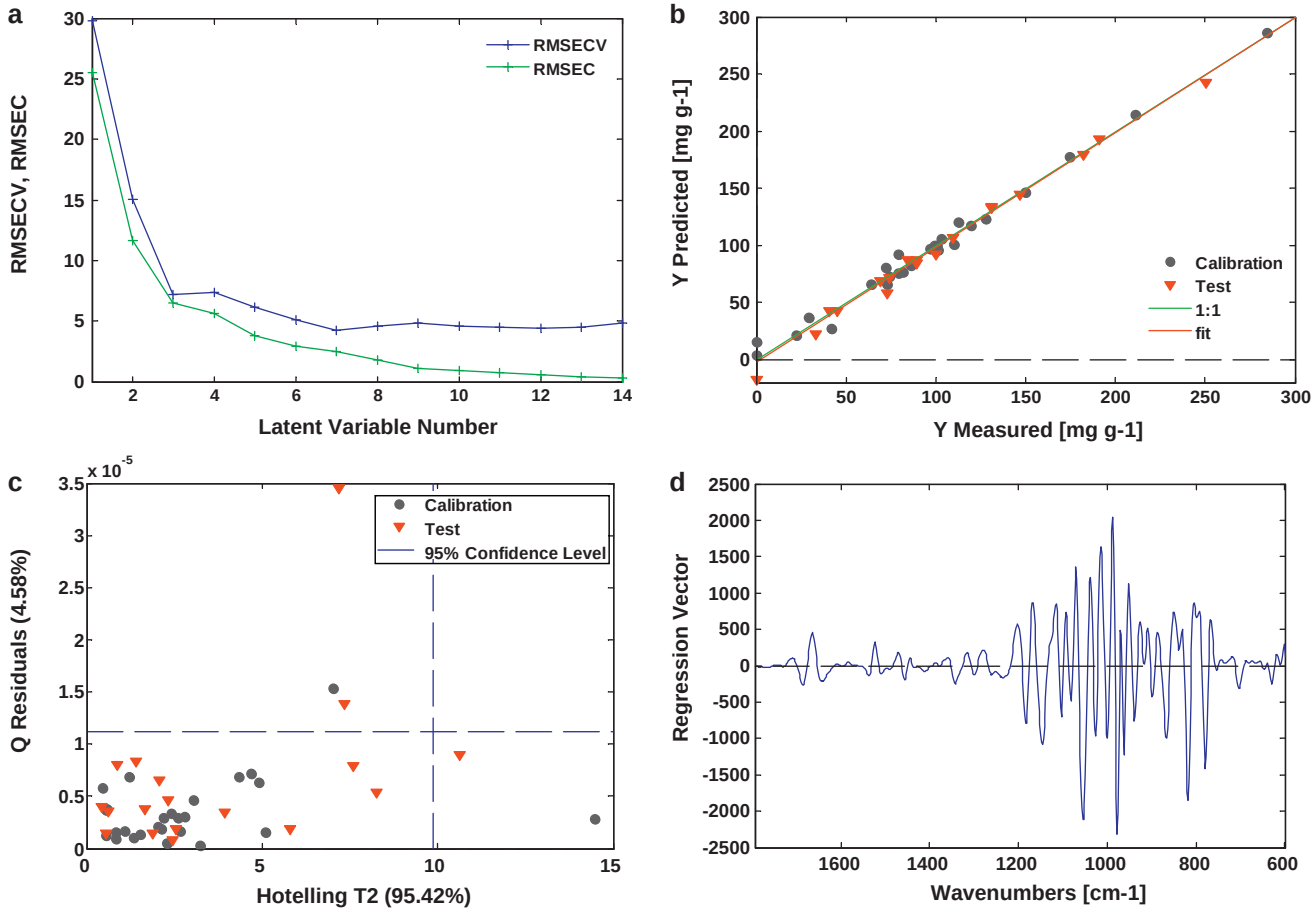

Fig. 4. Figures of merit of the ATR-FTIR-PLS model for the determination of fructose in depilatory formulations: (a) root mean square error of calibration (RMSEC) and cross validation (RMSECV) vs. the number of latent variables, (b) predicted vs. measured *y* values, (c) *Q* residuals vs. Hotelling T^2 values and (d) regression vector.

venetian blinds cross validation was carried out using 3 splits of the calibration data set.

The most important calibration and prediction parameters of the developed ATR-FTIR-PLS models are summarized in Table 4. Fig. 4 summarizes the results obtained for the PLS determination of fructose. According to the evolution of the root-mean-square-error of calibration (RSMEC) and cross validation (RMSECV) as a function of the number of latent variables (see Fig. 4a), three latent variables were selected, explaining 95.4% and 98.9% of the total variance of the **X** and **y** data blocks, respectively. In Fig. 4b, the calculated fructose concentration vs. the fructose concentration determined using the LC-IR method is depicted. All samples from the calibration and validation set follow the same trend close to the optimum *X*–*Y* line. The accuracy of the results is also reflected in the RMSEC, RMSECV and root-mean-square-error of prediction (RMSEP) showing similar values equal or lower than 7.17 mg fructose g^{-1} sample. The obtained bias values are acceptable and the coefficients of determination for calibration, cross validation and prediction indicate a high linearity with R^2 values equal or higher than 0.990. In Fig. 4c, the *Q* residual vs. the Hotelling T^2 values are shown. The Hotelling T^2 statistic is the sum of normalized squared scores and is a measure of the variation in each sample within the model. The *Q* residual is the sum of squares of each sample of the error matrix and therefore it is a measure of the difference, or residual, between a sample and its projection into the *k* latent variables space used to build up the model. It indicates how well each sample conforms to the PLS model [15].

It can be observed that both statistics are close to their optimum values of 0% and 100%, respectively, and only very few samples fall outside the 95% confidence limits. It has to be remarked, that one sample of the validation set was eliminated prior to the calculation of the final model because it showed anomalous *Q* residuals and Hotelling T^2 values. From the remaining data, no further samples could be clearly identified as outliers. Fig. 4d represents the regression vector, where the contribution of each variable to the PLS model can be observed. The range with the biggest influence is situated between 1200 and 800 cm^{-1}, thus confirming once again the appropriateness of the calibration model.

Fig. 5 shows the results of the model employed for the determination of glucose. Optimum results were achieved using 5 latent variables (see Fig. 5a), explaining 99.0% and 97.9% of the total variance of the **X** and **y** data blocks, respectively. The predicted values correspond to the concentrations determined using the reference procedure (see Fig. 5b), although the residuals are bigger than those obtained for the determination of fructose. This fact is also reflected in higher root-mean-square-errors, affecting in particular the RMSECV and RMSEP, resulting in values equal or lower than 12.55 mg glucose g^{-1} sample which are higher than those found for fructose but remain acceptable for this application. Bias values were comparable with those obtained for fructose and the coefficients of determination were also only slightly lower ($\geq$0.93). Fig. 5c shows the *Q* residuals and the Hotelling T^2 values for the samples used to calibrate and validate the glucose model. It has to be pointed out that a single data point of the calibration set and one data point of the validation set had to be eliminated prior to the calculation of the final model. These data points showed anomalous *Q* and Hotelling T^2 values. The regression vector (see Fig. 5d) also shows intense signals in the range between 1200 and 800 cm^{-1} although, at the

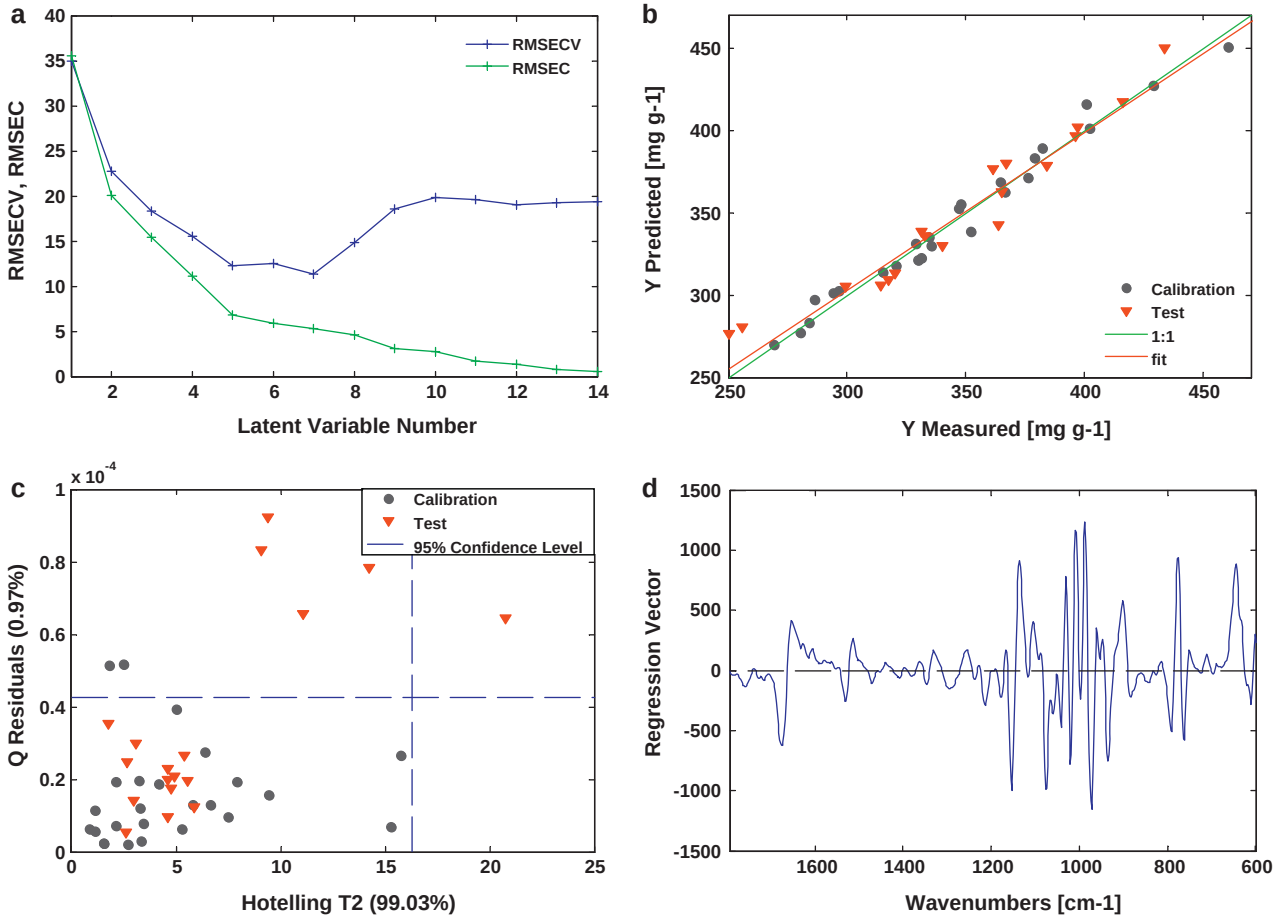

Fig. 5. Figures of merit of the ATR-FTIR-PLS model for the determination of glucose in depilatory formulations: (a) root mean square error of calibration (RMSEC) and cross validation (RMSECV) vs. the number of latent variables, (b) predicted vs. measured *y* values, (c) *Q* residuals vs. Hotelling T^2 values and (d) regression vector.

same time, other regions also seem to be of importance for the model.

The characteristics of the model obtained for maltose determination are summarized in Fig. 6. In this case, errors obtained from cross validation are much higher than those obtained for predictions. This might be caused by the chosen cross validation procedure (venetian blinds). Six latent variables were selected for the final model (see Fig. 6a), explaining 99.5% and 98.6% of the total variance of the **X** and **y** data blocks, respectively and adequate concentration values could be calculated for the calibration and validation set in comparison to those found by the reference LC-IR procedure (see Fig. 6b). Apart from a very high RMSECV, the RMSEC and RMSEP are of the same range as those obtained for fructose ($\leq$7.45 mg maltose g^{-1} sample). The same conclusions can be made concerning the bias values and the coefficients of determination ($\geq$0.990). The plot of the *Q* residuals vs. the Hotelling T^2 values (see Fig. 6c) confirmed that it is not necessary to eliminate any samples. The regression vector (see Fig. 6d) of the maltose model shows that the region between 1200 and 600 cm^{-1} is of importance.

In earlier studies PLS-ATR-FTIR spectroscopy has shown to be a useful tool for the determination of sugars in aqueous standards as well as in different complex matrices. However, the exact comparison of results obtained in different studies is impossible due to fundamental differences in the experimental set-ups and applications (e.g. use of different FTIR instruments, experimental measurement parameters, spectral regions, investigated concentration ranges and units, sample matrices, etc.). In order to compare the obtained results to existing methods, the RMSEP of different studies will be discussed in the following. The fructose and glucose concentrations in aqueous standards, using a concentration range in the validation set ranging from 1.5 to 6 g 100 mL^{-1} for both analytes, were determined resulting in mean relative prediction errors of 0.7% and 1.1%, respectively, employing optimized experimental conditions for each analyte [11].

The same analytes were determined in a complex matrix (apricot slurry) obtaining RMSEPs of 0.2 and 0.3 g sugar 100 g^{-1} sample, for fructose and glucose, respectively [8]. Considering that the concentrations of the validation samples ranged between 0.3 and 1.9 for fructose and between 0.7 and 4.9 g sugar 100 g^{-1} sample for glucose, the mean concentration values were 1.1 and 2.8 g sugar 100 g^{-1} sample, respectively. Consequently, calculating the RMSEP in %, errors of 13.6% and 9.3% were obtained for fructose and glucose.

In another study, the glucose concentration in different fermentations of *Escherichia coli* was determined. For two different validation sets with concentration intervals ranging from 6.65 to 9.98 and from 3.8 to 10.3 g L^{-1}, RMSEPs of 0.493 and 0.469 g L^{-1} were obtained, respectively. Referring these errors to the mean concentration of the validation samples, RMSEPs of 5.9% and 6.7% were obtained, respectively.

In the present study, the fructose concentrations ranged from 33.3 to 250.3, the glucose concentrations from 250.3 to 433.8 and the maltose concentrations from 25.3 to 149.2 mg sugar g^{-1} sample. Referring the obtained RMSEPs of 7.0, 12.6 and 7.5 mg sugar g^{-1} sample for fructose, glucose and maltose, respectively, to the mean concentration of the validation samples, results in errors of 5.0%, 3.7% and 8.5%, respectively. This comparison shows that prediction errors obtained in the present study were of the same order as prediction errors obtained by similar studies dealing with complex

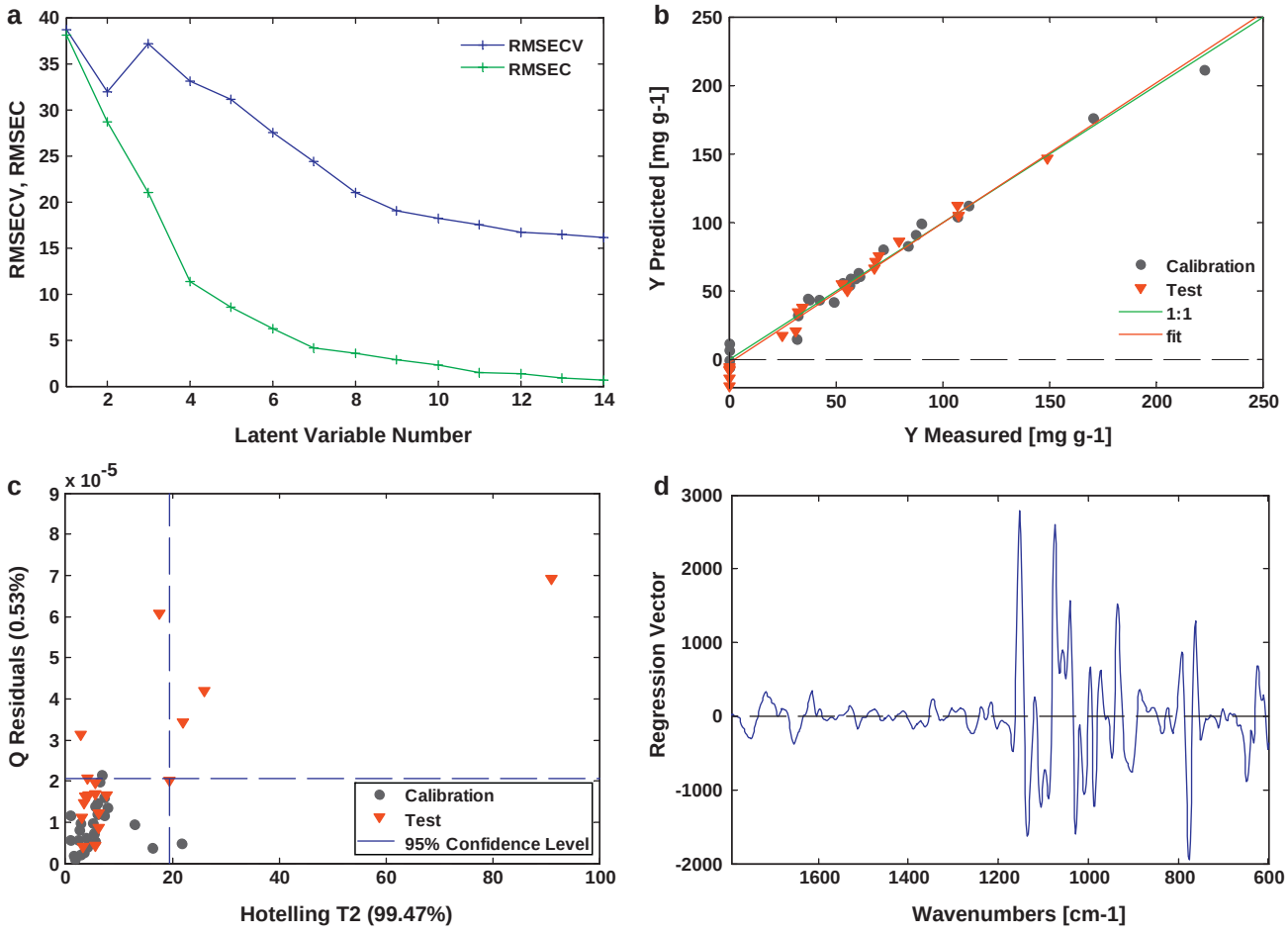

Fig. 6. Figures of merit of the ATR-FTIR-PLS model for the determination of maltose in depilatory formulations: (a) root mean square error of calibration (RMSEC) and cross validation (RMSECV) vs. the number of latent variables, (b) predicted vs. measured *y* values, (c) *Q* residuals vs. Hotelling T^2 values and (d) regression vector.

matrices. Only in comparison to the study were aqueous samples were analyzed, considerably lower prediction errors could be achieved.

4. Conclusions

The present paper reports the development of a direct method for the quantitative determination of fructose, glucose and maltose in depilatory formulations. To obtain reference concentration values of the analytes, in a first step, all samples were analyzed adapting a previously published on-line gradient LC-IR method. For background correction of on-line LC-IR data, an approach based on CSS [7] was used and linear calibration lines for fructose, glucose, sucrose and maltose were obtained. During the reference LC-IR analysis, important information on the composition of the samples was revealed. The absence of sucrose in all samples was evidenced, in spite of the information provided by the manufacturers ingredient lists. In fact, sucrose was added during the production process in all the cases, but due to acidic conditions and heating treatment of the primary matter during the production process, a cleavage of sucrose into fructose and glucose took place. Another remarkable observation was the detection of maltose in all samples, although apparently no maltose was added during the production process. The source of maltose might be glucose syrup which is frequently employed in the cosmetics industry instead of crystalline glucose. In summary, the sample compositions strongly differ from the information on ingredients indicated by the manufacturer, thus, evidencing the need of an analytical approach to improve the information provided to the costumers.

Employing the concentration data determined by the on-line LC-IR method as reference values, three PLS models for the quantification of fructose, glucose and maltose were calculated from the ATR-FTIR spectra of pure and mixed samples. After the selection of the spectral region and an appropriate spectral pre-treatment, RMSEP ranging between 7.04 and 12.55 mg sugar g^{-1} sample were obtained. This corresponds to relative prediction errors (referring to the mean concentration of the validation samples) of 5.0%, 3.7% and 8.5%, for fructose, glucose and maltose, respectively. In comparison to previously published studies dealing with the analysis of sugars in complex sample matrices, the obtained errors are of the same order. So, it can be concluded that the PLS treatment of ATR-FTIR data provided an accurate green tool for the determination of fructose, glucose and maltose in depilatories, being this methodology a direct, cheap and fast alternative to chromatographic approaches without requiring any sample pretreatment nor the use of solvents and reagents.

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