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# An infrared spectroscopic tool for process monitoring: Sugar contents during the production of a depilatory formulation

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ABSTRACT

A fast, reliable and economical methodology has been developed to control the production process of sugar-based depilatories. The method is based on the use of attenuated total reflectance—Fourier transform infrared (ATR-FTIR) spectroscopy in combination with multivariate data analysis. A very simple sample preparation process involving the dissolution of samples in water was applied. Employing a multivariate calibration model established from data of 15 well characterized samples, prediction errors equal or below 3.04 mg $mL^{-1}$ for the quantitative determination of fructose, glucose, sucrose, maltose and maltotriose were obtained. Results found in this preliminary study indicate a great potential for the development of at-line ATR-FTIR-PLS methods based on a careful selection of variables from IR spectra, delivering fast and reliable results. As a reference method, a liquid chromatography (LC)–IR method was adapted for sample characterization.



## 1. Introduction

Process analytical technology (PAT) continues to be an evolving field across several industrial sectors reflected by an ongoing interest in the development and implementation of new analytical tools and methods. Frequently the evaluation of product quality is accomplished by analyzing randomly collected samples obtained from batch processing, employing time-consuming off-line laboratory techniques. This lack of understanding of the process itself hampers effective process control and can result in batch losses. In accordance, the objective of PAT is to enhance understanding and control a manufacturing process following quality-by-design principles, to reduce identified manufacturing risks that are associated with product quality. It should therefore play a crucial role in design, analysis and control of manufacturing processes based on measurements of critical performance attributes [1–3].

Concerning available analytical tools, modern technologies providing multivariate information related to biological and chemical parameters have evolved from those that predominantly take univariate process measurements, such as temperature, pH and pressure. In the last decade, vibrational spectroscopy has been increasingly used for process control and monitoring in many different fields including the monitoring of pharmaceutical [4,5] and bio-processes [6]. The huge number of methods based on different spectroscopic techniques such as mid-infrared (MIR), near infrared (NIR) and Raman spectroscopy can be attributed to their characteristics that make them well-suited for process control and monitoring tasks combining several key-features. In general, spectroscopic methods are able to provide quantitative and qualitative multi-analyte information being at the same time extremely versatile as they allow the measurement of gaseous, liquid and solid samples. Further remarkable aspects that favor the implementation of spectroscopic techniques are that measurements are usually non-destructive and sometimes even non-invasive. The set-ups are robust and economic and ideally allow the implementation of automated data acquisition in real-time measurements. Instrumentation technology for process analytical applications can avoid the deleterious side effects of traditional methods involving an intensive sample preparation and, because of that green analytical procedures have been replacing other PAT [7,8].

Due to the frequent use of vibrational spectroscopic process analyzers and their ability to supply multivariate information, there is a growing need for chemometric methods. Currently, all kinds of multivariate regression and classification models find their application in (bio-)process monitoring [1,8–10] and methods are constantly developed to improve results [11].

Although similar from the viewpoint of an analytical chemist, in contrast to the pharmaceutical sector, PAT still plays a limited

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role in the production of cosmetics. Usually, only final products are analyzed employing standard laboratory techniques to ensure consumer safety according to the EU legislation on cosmetic products [12–14]. On the other hand, IR spectroscopy is known to be a valuable tool for the determination of glucose in complex samples like blood and urine [15]. Furthermore recently a method for the direct determination of fructose, glucose, sucrose and maltose in sugar based depilatories employing ATR (attenuated total reflectance)-FTIR spectra was developed as an alternative to solvent and time-consuming chromatographic methods [16]. Surprisingly, in this study the absence of sucrose in all tested commercial samples was evidenced in spite of the information on components provided by the manufacturers. In fact, sucrose was added during the production process, but due to heating of the primary matter under acidic conditions, a cleavage of sucrose into fructose and glucose was suspected. Moreover maltose was detected in all analyzed samples although apparently no maltose was added during production, which raised the suspicion that glucose syrup instead of crystalline glucose was added.

Based on the foregoing study, in the present study the previously developed liquid chromatography (LC)-IR method was adapted to analyze samples withdrawn at different stages of the production process to gain a deeper insight into changes of raw materials occurring during the production. After sample characterization a direct, green ATR-FTIR method for the simultaneous determination of all sugars present during the batch production process of depilatories was developed as a preliminary study for its implementation as at-line PAT.

## 2. Material and methods

### 2.1. Standards and samples

D(−)-fructose, anhydrous D(+)-glucose and maltose-1-hydrate from Panreac (Barcelona, Spain), D(+)-sucrose from Scharlau (Barcelona, Spain) and maltotriose hydrate 95% from Aldrich (Saint Louis, MO, USA) were used as standards. Acetonitrile (HPLC grade) was purchased also from Scharlau and high-purity water, with a resistivity higher than 18.2 MΩ, was obtained from a Milli-Q water-purification system (Bedford, MA, USA). Ten different stages of the batch process were considered, characterized by the addition of sugars and perfume and a temperature program. Aliquots of approximately 3 mL were withdrawn from the batch reactor at each production step and stored until analysis in glass vials in the dark at room temperature.

### 2.2. LC-IR reference procedure

Recently published LC-IR methods for the determination of sugars were adapted [16,17] for the off-line analysis of samples withdrawn from the production process. For chromatographic separations a Dionex (Sunnyvale, CA, USA) P680 high performance liquid chromatography system, equipped with a Kromasil 100 $NH_2$ column (250 × 2 mm, 5 μm) was used running linear acetonitrile:water gradients from 75 to 55% (v/v) of acetonitrile in 15 min, then maintaining the mobile phase constant during 10 min at a flow rate of 0.2 mL $min^{-1}$. Using a home-made micro flow cell interface with an optical path of 10 μm, consisting of a ZnSe and a $CaF_2$ window and an aluminium spacer, on-line hyphenation to a Bruker IFS 66/v FTIR spectrometer (Ettlingen, Germany) was achieved. Post-run background correction was carried out employing cubic smoothing splines (CSS) [18] and linear calibration lines were established by measuring the corresponding peak areas obtained from the analysis of standards.

For preparation of sugar standard mixtures, different amounts of pure sugar standards were accurately weighed in 5 mL volumetric flasks and dissolved in 2.5 mL of Milli-Q water. Standard mixtures were sonicated in a JP Selecta ultrasonic water bath (Barcelona, Spain) during 25 min and filled up to volume with acetonitrile. For sample preparation, between 200 and 250 mg of each sample were accurately weighed in a 5 mL volumetric flask following the same procedure as described for standard solutions. Sample solutions were centrifuged at 2500 rpm during 15 min to eliminate un-dissolved particles. Before injection into the chromatographic system, standard and sample solutions were filtered through 0.22 μm nylon syringe filters.

### 2.3. ATR-FTIR-partial least squares (PLS) procedure

For ATR spectra acquisition, a dry-air purged in-compartment DuraSampleIR accessory from Smiths Detection Inc. (Warrington, UK) equipped with a nine reflection diamond was installed on a Bruker IFS 66/v FTIR spectrometer. Spectra were recorded in the range between 4000 and 600 $cm^{-1}$, with a spectral resolution of 4 $cm^{-1}$, averaging 100 scans per spectrum and using a spectrum of Milli-Q water as a background.

For sample preparation of pure samples, 1.5 mL of Milli-Q water were added to 200 mg of each sample. In addition to the pure samples, 31 mixed samples were prepared as binary mixtures of the pure ones by accurately weighing different amounts of two samples ranging between 150 and 300 mg and adding 1.5 mL of water. The dilution step was necessary because of the high viscosity of samples hindering a proper handling of the aliquots, and enabled at the same time the preparation of homogeneous mixed samples. All samples were sonicated in an ultrasonic water bath during 25 min. Sugar concentrations of pure and mixed samples were calculated employing concentrations determined by the on-line LC-IR reference procedure and the weighed sample masses.

Triplicate spectra were obtained for each sample and sample mixture by depositing an aliquot onto the ATR crystal. The means of the triplicate spectra of each sample and/or sample mixture were calculated and employed to build up a calibration and a validation subset for PLS modeling, containing 15 and 26 spectra, respectively (for details see Table S1 of Suplementary Material). Table 1 describes the main characteristics of the data sets. Prior to PLS model calculation, mean centered row vectors resulting from 9-point cubic Savitzky–Golay first or second derivative spectra were calculated in order to improve PLS calibration. Derivative spectra are commonly used for pre-processing of spectroscopic data in order to remove baseline drifts and offsets. They remove low-frequency features maintaining high-frequency features that contain the signal of interest without affecting the linear relationship with the chemical concentration [19]. Leave-one-out cross validation (CV) was employed for the calculation of internal figures of merit and for the selection of the optimum number of latent variables (LVs). For outlier detection, the Hotelling $T^2$ statistic and $Q$ residuals were calculated. The Hotelling $T^2$ statistic is a measure of the variation in each sample within the model defined as the sum of normalized squared scores. The $Q$ residuals are the sum of squares of the error matrix of each sample. The $Q$ residual is a measure of the difference between a sample and its projection into the latent variables space used to build up the PLS model and therefore it indicates how well each sample conforms to the PLS model [19].

A strategy to improve PLS regression model performance is to select one or more spectral ranges containing useful chemical information and to eliminate spectral ranges only contributing noise. A preliminary variable selection was carried out using interval PLS (iPLS), because it has several intrinsic advantages over other variable selection methods: (i) the interpretation of the

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

| Set | Type of samples | Number of samples | Parameter [mg mL$^{-1}$] | Fructose | Glucose | Sucrose | Maltose | Maltotriose |
|---|---|---|---|---|---|---|---|---|
| Calibration | Pure and mixed samples | 10+5 | Mean value | 11.6 | 39.4 | 6.2 | 12.7 | 6.0 |
| | | | Minimum value | 1.2 | 4.9 | 0.0 | 5.8 | 0.0 |
| | | | Maximum value | 25.3 | 74.4 | 27.8 | 20.8 | 11.7 |
| | | | Standard deviation | 6.3 | 17.5 | 8.9 | 4.5 | 3.6 |
| Validation | Mixed samples | 26 | Mean value | 12.7 | 40.7 | 7.7 | 13.8 | 6.6 |
| | | | Minimum value | 5.1 | 18.5 | 0.0 | 7.6 | 0.0 |
| | | | Maximum value | 23.0 | 69.9 | 22.3 | 23.1 | 13.3 |
| | | | Standard deviation | 5.5 | 15.6 | 8.5 | 4.4 | 3.2 |

*Note*: for PLS modeling, one sample had to be eliminated from the calibration set of fructose; for validation of the glucose model, one sample had to be eliminated from the validation set.

results is straightforward even for inexperienced users as whole intervals and no single variables are selected, (ii) no high computation power is necessary for its calculation in contrast to more sophisticated methods like e.g. genetic algorithms or artificial neuronal networks and (iii) reproducible results are achieved in comparison to other variable selection tools like e.g. uninformative variable elimination (UVE)-PLS. The iPLS variable selection is based on the calculation of local PLS sub-models of a user defined number of equidistant variable intervals applying a range of LVs. Root mean square errors of cross validation (RMSECV) from local PLS sub-models were compared for variable selection removing those intervals providing high RMSECVs [20]. In this study, iPLS models were built up using the same pre-processing and cross validation procedure as for PLS model calculation and dividing the spectra into 10 independent intervals, using a maximum of 10 latent variables in each interval.

To optimize results obtained from iPLS, PLS models were calculated using the selected intervals and calculating the Variable Importance in Projection (VIP) scores. Their calculation is based on estimating the importance of each variable in the projection used in a PLS model in such a way that a variable with a VIP score higher than one can be considered important in a given model [19]. VIP scores allow a straightforward evaluation of spectral regions with a high contribution to the model even when derivatives were employed during pre-processing.

### 2.4. Software and algorithms

The chromatographic system was controlled employing Chromeleon 6.40 from Dionex (Sunnyvale, CA, USA) and Opus 6.5 from Bruker was used for instrumental and measurement control of the IR spectrometer as well as for data acquisition. Background correction was carried out using in-house written functions running in Matlab 7.7.0 from Mathworks (Natick, MA, USA). For PCA and PLS model calculation and validation, the PLS Toolbox 6.5 from Eigenvector Research Inc. (Wenatchee, WA, USA) was used and for iPLS model calculation, the iPLS Toolbox was employed [21], both running in a Matlab environment. Detailed information on PLS regression and other related parameters employed in this work have been discussed earlier [22,23].

## 3. Results and discussion

### 3.1. Investigation of the production process employing on-line LC-IR

In a previous study analyzing different depilatory products, an enormous difference between the raw materials declared by the manufacturers and the sugar concentrations in the final product was found. As the sugar composition determines product properties, for product development and quality assurance it is of interest to control the concentration of all sugars during batch production. For this reason, the evolution of sugar concentrations during the process should be monitored.

An existing on-line LC-IR method for the analysis of fructose, glucose, sucrose and maltose was extended to the analysis of maltotriose. External calibration lines for all analytes were established from the injection of sugar standard mixtures by means of linear regression of the chromatographic peak areas vs. the analyte concentration. After applying a background correction algorithm, traces for fructose, glucose as well as sucrose, maltose and maltotriose displayed in Fig. S1 (top) could be extracted using absorbance measurements at 1069, 1080 and 1065 cm$^{-1}$ and a single point baseline correction at 1204, 1184 and 1177 cm$^{-1}$, respectively. Although retention time shifts due to strongly varying temperature conditions between injections were observed, all five analytes under study could be clearly identified by their IR spectra (data not shown). Table 2 shows the figures of merit of all five LC-IR calibration lines obtained. The limits of detection and quantification (LOD and LOQ) as well as the repeatability were acceptable for the determination of sugars in depilatories, because the analysis of major compounds that are usually present at high concentration levels are concerned.

During batch production, ingredients were added at different stages of the process and the mixture was heated. First, glucose syrup was moderately heated (steps 1 and 2). Then temperature was increased and sucrose was added (step 3). After the addition of citric acid, the mixture was maintained at different temperature levels (all high temperatures, steps 4 to 8) during several hours before glucose and glycerol (step 9) and later on perfume (step 10) were added at moderate temperatures. All samples withdrawn at different stages of the process were analyzed with the on-line LC-IR system employing the same conditions as for standard mixtures. The obtained chromatograms of some samples considered representative of the process are depicted in Fig. S1.

In Fig. 1 determined sugar concentrations observed during the production process are shown. It can be seen that initially fructose, glucose, maltose and maltotriose were detected in the glucose syrup. Fructose was expected to be observed as the employed raw material declared to contain fructose. For the production of glucose syrup, acidic or enzymatic hydrolysis of starch is employed resulting in dextrose (D-glucose monosaccharide), maltose (glucose di-saccharide), maltotriose (glucose tri-saccharide) and high molecular weight saccharides [24], explaining the observed sugar concentrations. When sucrose was added, relative concentrations of other sugars drop due to dilution effects. Sucrose is hydrolyzed due to heat treatment and acidic pH [25] and already in the fourth sample step no sucrose could be detected, whereas increased concentrations for glucose and fructose were determined. Between steps four and eight little

**Table 2**
Figures of merit of the on-line LC-IR determination of sugars.

| Analyte | Concentration range [mg ml$^{-1}$] | $y=a\pm S_a+(b\pm S_b)x$[a] | | $R^2$ | LOD [mg ml$^{-1}$][b] | LOQ [mg ml$^{-1}$][c] | Repeatability [%][d] |
|---|---|---|---|---|---|---|---|
| | | $a\pm S_a$ | $b\pm S_b$ | | | | |
| **Fructose** | 3.4–10.0 | $-0.02\pm0.02$ | $0.392\pm0.005$ | 0.998 | 0.8 | 2.7 | 6.9 |
| **Glucose** | 3.3–23.1 | $0.14\pm0.06$ | $0.317\pm0.007$ | 0.995 | 0.4 | 1.2 | 11.5 |
| **Sucrose** | 3.5–10.2 | $0.02\pm0.06$ | $0.420\pm0.014$ | 0.990 | 0.5 | 1.6 | 5.5 |
| **Maltose** | 3.3–16.3 | $0.03\pm0.04$ | $0.264\pm0.005$ | 0.996 | 0.4 | 1.2 | 6.7 |
| **Maltotriose** | 3.9–10.3 | $0.08\pm0.09$ | $0.371\pm0.016$ | 0.990 | 0.5 | 1.6 | 8.9 |

Note:
[a] Calibration curve from 6 standard solutions; *a* and *b* are the intercept and the slope of the calibration lines.
[b] Limit of detection established as three times the standard deviation of four independent measurements at a concentration level of 3 mg ml$^{-1}$ divided by the analytical sensitivity.
[c] Limit of quantification established as ten times the standard deviation of four independent measurements at a concentration level of 3 mg ml$^{-1}$ divided by the analytical sensitivity.
[d] Relative standard deviation for four independent measurements carried out at a concentration level of 3 mg ml$^{-1}$.

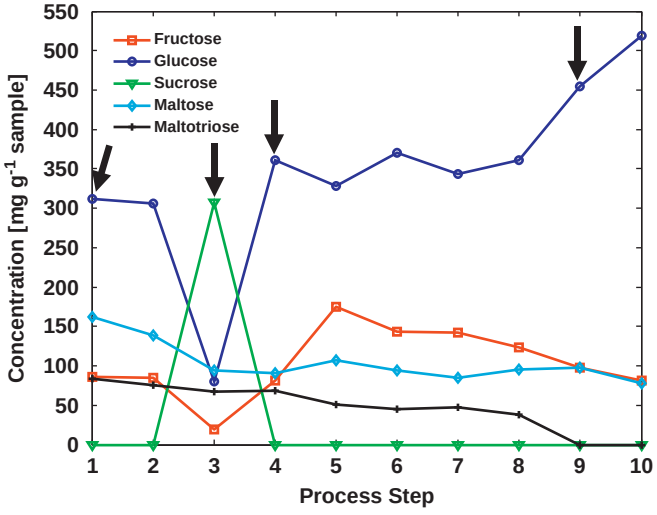


**Fig. 1.** Evolution of sugar concentrations during production process of sugar-based depilatories. *Note*: arrows indicate the incorporation of glucose syrup in step 1, sucrose in step 3, citric acid in step 4 and glucose and glycerol in step 9.

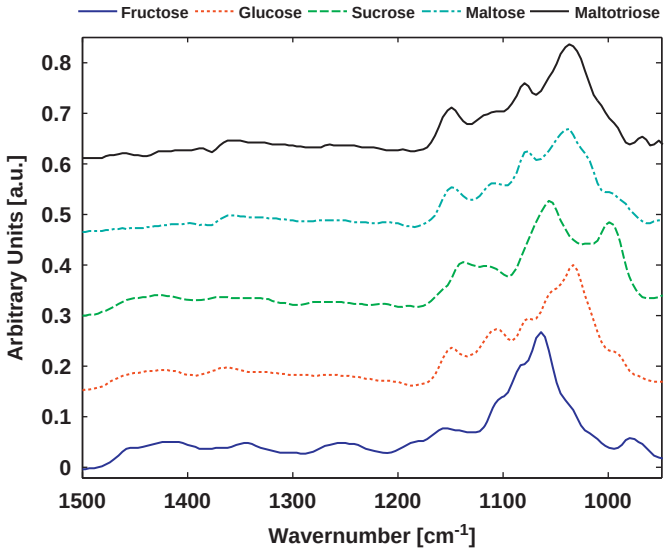


**Fig. 2.** Spectra of fructose, glucose, sucrose, maltose and maltotriose measured using a flow cell. *Note*: Spectra have been normalized to maximum absorption and shifted along the *y*-axis for a better visibility.

variation in all sugar concentrations was observed. In accordance with the addition of glucose and glycerol towards the end of the process, raising glucose concentrations were detected. The concentration of maltotriose declines during the process which is probably due to the conversion into maltose and glucose.

### 3.2. Development of a direct ATR-FTIR-PLS method for process monitoring of sugar concentrations

Fig. 2 shows spectra of standards of five pure analytes dissolved in water in the region between 1500 and 950 cm$^{-1}$ where the analytes show typical absorbance bands. A detailed discussion on mid IR bands of carbohydrates is out of the scope of this work and can be found elsewhere [26–28]. Briefly, the studied compounds show strongly overlapping absorption bands mainly caused by C–O and C–C stretching and C–OH deformation modes in the depicted region. Additionally, bands in the region around 3000 cm$^{-1}$ are observed caused by $CH_2$ and $CH_3$ stretching vibrations. From Table 3 it can be appreciated that all analytes under investigation show very similar signals in the region between 1500 and 950 cm$^{-1}$. This fact evidences that an accurate determination of all five investigated analytes requires the use of multivariate statistical tools since univariate methods are not powerful enough.

**Table 3**
Correlation coefficients calculated between reference spectra of the different analytes shown in Fig. 2 in the spectral interval between 1500 and 950 cm$^{-1}$ (italic numbers) and between the concentration vectors of the spectra used for PLS model calibration (bold numbers).

| | Fructose | Glucose | Sucrose | Maltose | Maltotriose |
|---|---|---|---|---|---|
| **Fructose** | 1 | *0.8* | *0.7* | *0.8* | *0.8* |
| **Glucose** | **0.4** | 1 | *0.90* | *0.97* | *0.97* |
| **Sucrose** | **0.5** | **0.6** | 1 | *0.90* | *0.90* |
| **Maltose** | **0.1** | **0.08** | **0.03** | 1 | *0.990* |
| **Maltotriose** | **0.004** | **0.1** | **0.03** | **0.5** | 1 |

Fig. 3a shows ATR-FTIR spectra of samples withdrawn from 10 different steps of the production process as indicated in the experimental part and Fig. 3b shows the same spectra after subtracting the spectrum of the first sample. From the figure it can be seen that spectra of all samples are highly similar, with some minor variations in the band around 1050 cm$^{-1}$, caused by differences in the carbohydrate composition. Comparing this figure to results shown by Cascant et al. [16], for samples directly measured without the addition of water, it can be observed that similar signals were obtained for the depilatory samples.

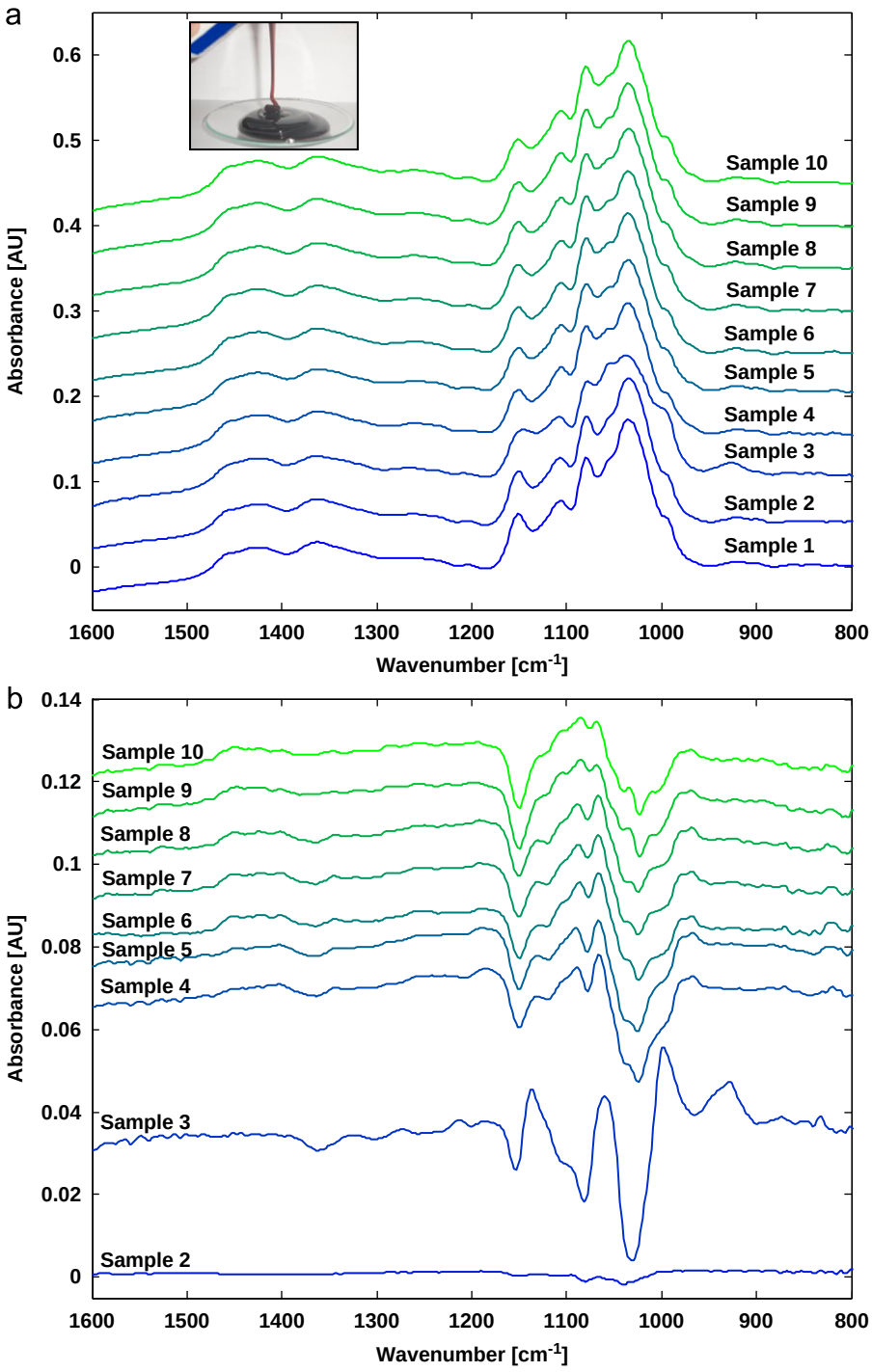


**Fig. 3.** (a) ATR-FTIR spectra of samples withdrawn at different stages of the production process and (b) difference spectra of the same samples as shown in (a) subtracting the spectrum of the first sample. *Note*: sample spectra were normalized to maximum absorption and shifted along the y-axis for a better visibility. *Note*: Insert in Fig. 3a shows a picture of a representative sample.

This confirms that water did not cause significant interferences in the acquired signal as its contribution could be accurately compensated by using a water spectrum as a background.

Due to the high similarities in the signals of the analytes it is important to provide calibration spectra with independently varying carbohydrate concentrations for PLS modeling. The correlation coefficients of the concentration vectors employed for the calibration set are displayed in Table 3 and are always below or equal to 0.6.

Fig. S2 displays results obtained from iPLS variable selection for fructose, glucose, sucrose, maltose and maltotriose. For all five models, the interval between 1280 and 940 $cm^{-1}$ gave low RMSECV. For the majority of the models, also the adjacent region from 1620 to 1280 $cm^{-1}$ added useful information reflected in low RMSECV values. As a rough estimation, both intervals were employed for PLS modeling of fructose, glucose, sucrose, maltose and maltotriose. The selection of these regions is in agreement with typical absorbance bands of carbohydrates as discussed above. Furthermore, based on the results provided by the iPLS algorithm, the region around 3000 $cm^{-1}$ can be excluded from further calculations, as it is not considered to add useful information for PLS modeling (with exception of maltose), although all

analytes present bands in this region. The results demonstrate, that iPLS is a valuable tool for straightforward variable selection, without the need of expert knowledge for the a priori exclusion of regions like the region around 2100 $cm^{-1}$, where high noise levels caused by the limited transparency of the diamond of the ATR unit are observed.

Employing the variables between 1620 and 940 $cm^{-1}$ and the previously selected spectral pre-processing (see Table 4), five PLS models were calculated and a plot of the VIP scores was obtained as shown in Fig. 4 using the example of glucose. It can be clearly observed that mainly variables in the region between 1180 and 960 $cm^{-1}$ are contributing to the model. Absorbance bands in sugar spectra in the considered spectral region are attributed to strongly overlapping C–O and C–C stretching and C–OH deformation vibrations shown in the inset of Fig. 4. As similar results were obtained for all analytes, finally the same region could be employed to build all five models.

### 3.3. Analytical figures of merit of the developed procedure

Fig. 5 shows the results obtained for the PLS determination of fructose, glucose, sucrose, maltose and maltotriose. According to the root-mean-square-errors of cross validation, the optimum number of latent variables was chosen as indicated in Table 4. Predicted values of sugar concentrations vs. sugar concentrations determined using the LC-IR reference method are depicted, along with the $Q$ residual vs. the Hotelling $T^2$ values. From Fig. 5 it can be concluded that calibration and validation sample points were closely distributed near the optimum regression line between measured and predicted concentrations. The Hotelling $T^2$ values and $Q$ residuals in the insets of Fig. 5 evidence that no clear outliers could be identified, as only very few samples fall outside the 95% confidence limits and both statistics are close to their optimum values of 100% and 0%, respectively. It has to be remarked that one sample had to be removed from the calibration set of fructose and one sample from the validation set of glucose prior to the calculation and validation of the final PLS models of those analytes.

The calibration set of sucrose appears to be imbalanced due to the high number of calibration samples with a zero concentration caused by the fact that sucrose is only present in the production process during a very short time period. However, when calculating a PLS model after eliminating six samples with zero concentration of sucrose, it could be proved that the performance of the PLS model was not significantly influenced (data not shown). Hence, the use of the present data set was preferred for assuring a straightforward application of ATR-FTIR in combination with PLS calibration as a process analytical tool, using the same calibration set for the determination of all five considered analytes.

The most important calibration and prediction parameters of the developed ATR-FTIR-PLS models are summarized in Table 4. As shown in Table 4 either the first or second derivative was applied prior to PLS modeling. Derivatives are a commonly used pre-processing step in PLS model calculations when working with spectroscopic data, where adjacent variables are inherently related to each other containing similar correlated signals. They are a form of high-pass filters used to remove unimportant baseline signals. A first derivative effectively removes any offset from the sample and de-emphasizes low-frequency signals whereas a second derivative, calculated by repeating the process, will further accentuate high-frequency features. It is important to remark that the calculation of derivatives does not affect any linear relationships within the data. The choice between first and second derivative has to be made in order to achieve optimum PLS modeling parameters for the calibration data, confirming the suitability of the chosen pre-processing employing an external validation set. A first derivative was preferred over a second derivative, as using the second derivative the noise is accentuated

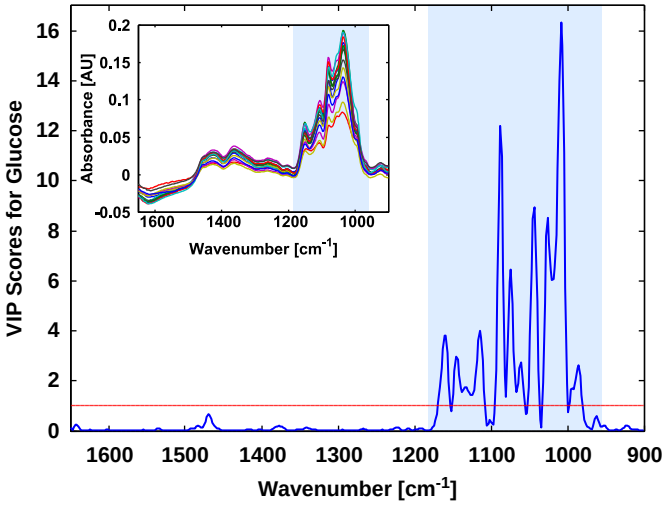


**Fig. 4.** VIP scores calculated for optimizing the spectral regions employed for PLS modeling of ATR-FTIR spectra to build models for carbohydrate determinations, using the example of glucose. *Note*: the inset shows ATR-FTIR spectra included in the calibration set.

**Table 4**
Calibration and prediction parameters of the ATR-FTIR-PLS models developed for the determination of fructose, glucose, sucrose maltose and maltotriose at different steps of a depilatory production process. *Note*: the spectral interval used for all models was from 1180 to 960 $cm^{-1}$.

| Analyte | Pre-processing | LVs[a] | RMSECV[b] [mg $mL^{-1}$] | RMSEP[c] [mg $mL^{-1}$] | CV[d]Bias [mg $mL^{-1}$] | Pred[e]Bias [mg $mL^{-1}$] | $R^2$ Cal[f] | $R^2$ CV[d] | $R^2$ Pred[e] |
|---|---|---|---|---|---|---|---|---|---|
| **Fructose[g]** | 2nd Derivative | 3 | 2.81 | 2.94 | −0.26 | 1.44 | 0.96 | 0.80 | 0.82 |
| **Glucose[h]** | 1st Derivative | 3 | 4.06 | 3.04 | −0.39 | 2.00 | 0.98 | 0.94 | 0.98 |
| **Sucrose** | 1st Derivative | 4 | 0.61 | 0.65 | 0.06 | −0.16 | 0.998 | 0.995 | 0.996 |
| **Maltose** | 1st Derivative | 3 | 1.59 | 0.90 | −0.02 | 0.02 | 0.95 | 0.90 | 0.96 |
| **Maltotriose** | 2nd Derivative | 4 | 1.14 | 0.66 | −0.19 | 0.16 | 0.98 | 0.90 | 0.97 |

Note:
[a] LVs: latent variables,
[b] RMSECV: root mean square error of cross validation,
[c] RMSEP: root mean square error of prediction,
[d] CV: cross validation,
[e] Pred: prediction,
[f] Cal: calibration,
[g] Fructose: one sample eliminated from calibration set,
[h] Glucose: one sample eliminated from validation set.

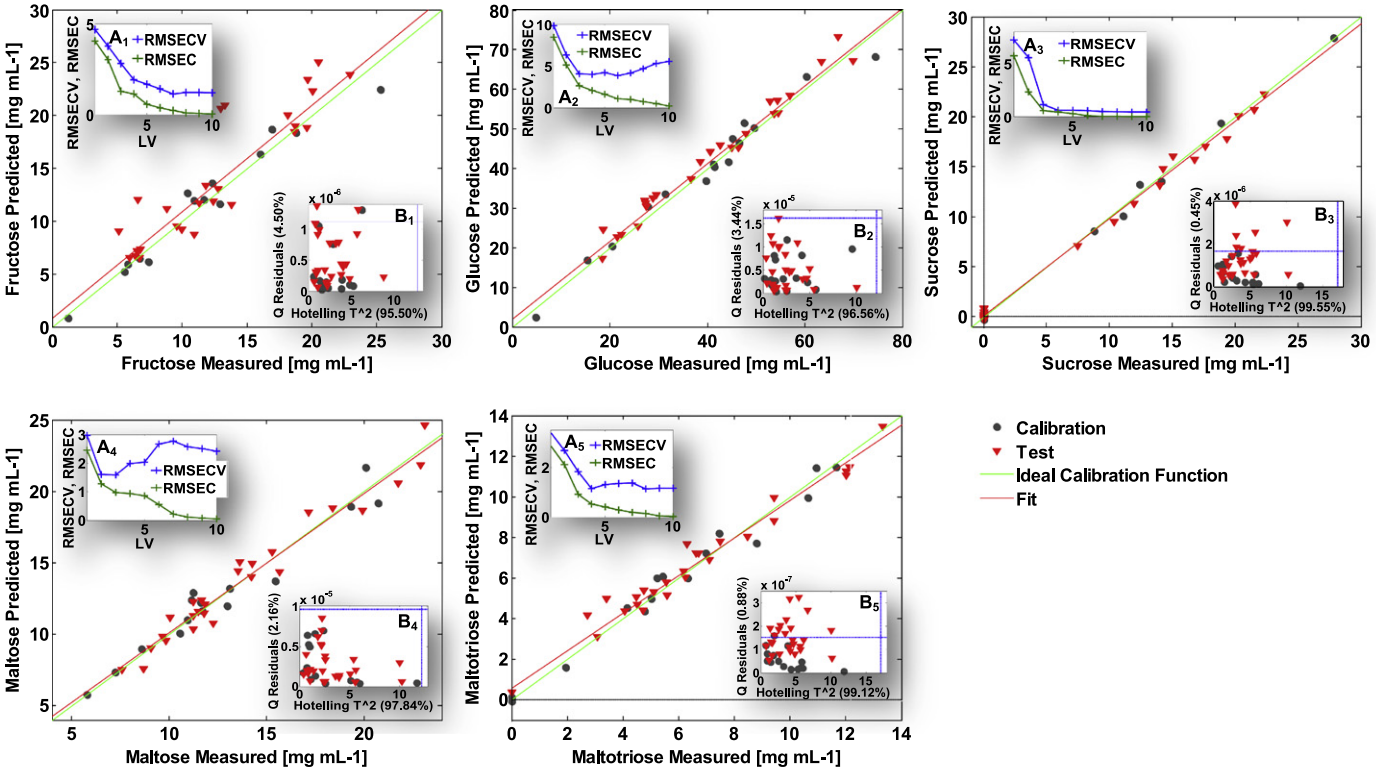


**Fig. 5.** Prediction capabilities of the ATR-FTIR-PLS models for the determination of fructose, glucose, sucrose, maltose and maltotriose in samples withdrawn from a depilatory production process. Insets: (A) Evolution of the root mean square error of calibration (RMSEC) and cross validation (RMSECV) as a function of the number of latent variables (LVs). (B) *Q* residuals vs. Hotelling $T^2$ values obtained for each model. *Note*: blue dashed lines represent the 95% confidence level. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

stronger. Second derivatives were applied only in cases where the discrimination/resolution enhancement provided by the use of the first derivative was not sufficient.

Acceptable cross validation and prediction errors below or equal to 4.06 mg $mL^{-1}$ of sugar were achieved in all cases. Within each model similar values for the RMSECV and root-means-square error or prediction (RMSEP) were obtained, indicating good model performance. Low cross validation and prediction bias values, as well as high correlations of determination ($R^2$) supported the quality of model performance. Only in the case of fructose, slightly lower $R^2$ values were observed. However, concerning the application of this method as a process analysis tool, the overall model performance was considered to be adequate.

Regarding green analytical parameters [29], sample preparation only requires the use of hundred mg amounts of sample dissolved in water thus, the use of organic solvents, like acetonitrile, is avoided reducing both, the cost and environmental impact of the analytical procedure. On the other hand direct recording of ATR spectra without any previous analyte separation improves the sampling throughput, reduces energy consumption and avoids the use of LC instrumentation. So, the main strengths of the green method proposed here consist of the lack of toxic and dangerous reagents, the reduced amounts of samples required and the absence of toxic residues together with the high capability of the procedure for simultaneous monitoring of sugars during different steps of depilatory production. The opportunity offered by the proposed methodology involves the green process monitoring of cosmetics being only limited by the need of a series of samples, well characterized by a reference chromatographic procedure, to correctly build the PLS models.

The main drawback of the method is the use of aqueous solutions of samples for ATR measurements which avoids the implementation of this method in on-line or in-line process monitoring. However, in this case at-line measurements present an advantage in comparison to in-line and non-invasive measurements as the process includes different temperature steps which can strongly influence the spectral data.

## 4. Conclusions

For controlling product properties of sugar-based depilatories it is of utmost importance to determine the sugar composition during production. Samples were withdrawn from different stages of the production process and analyzed by a reference on-line LC-IR laboratory method, thus providing quantitative data on fructose, glucose, sucrose, maltose and maltotriose at different steps of the process. This helps gaining insight into the process of sucrose and maltotriose hydrolysis and provides well characterized samples, avoiding unknown interferences, for building the corresponding ATR-FTIR-PLS models. The careful variable selection from the ATR-FTIR signals of different samples allowed the construction of PLS models for the quantification of fructose, glucose, sucrose, maltose and maltotriose resulting in RMSEP below or equal to 3.04 mg $mL^{-1}$.

Comparing the time necessary for sample preparation and analysis, the chromatographic run-time per sample using the reference LC-IR method is about 30 min, including a 5 min waiting time to achieve stable conditions within the flow cell interface after each gradient run. Sample preparation necessary for chromatographic analysis took about 40 min, including 25 min for sonication and 15 min for centrifugation, although it has to be taken into account that several samples can be prepared at the same time. In contrast, the measurement time employed using the proposed ATR-FTIR procedure is about 3 min, including 1 min for the acquisition of three replicate spectra and approximately

2 min for cleaning the ATR crystal. Sample preparation is also less time consuming, as the centrifugation step is not necessary. Without taking into account the calibration procedure, this leads to a total saving of time during sample preparation of almost 40% and provides a ten times faster analysis.

This work can be viewed as a preliminary study for the development of at-line ATR-FTIR-PLS methods employing standard process IR instrumentation for obtaining quantitative multi-analyte information. Complex sample preparation was avoided and the good performance of a very simple calibration approach based on the measurement of only 15 calibration standards could be shown. This technique could therefore be potentially employed as a fast and environmentally friendly alternative to chromatographic analysis methods for PAT.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2012.06.072.

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