UNORTHODOX FAST ANALYSIS OF BISPHENOLS S AND F IN PHENOLIC SYNTANS

Luca Iannarelli, Skerdi Korri, Alessia Pensato, Fabio Romanazzi, Costanza Aghemo

R&D Department, I.C.A.I. S.p.A., Viale Cruto 27, 10090, Bruino, Turin Metropolitan Area, Italy, Tel: 0119048260; c.aghemo@icai.it

ABSTRACT

It is a truth universally acknowledged that bisphenols are the essential building blocks for a whole category of tanning and retanning agents. Their unique characteristics allow to obtain full leather with high levels of softness, fastness and whiteness. In the last period, many attentions from different sectors, including leather one, were focused on bisphenol S (BPS) and bisphenol F (BPF) because of their potential adverse effects on human health, as well as bisphenol A (EFSA 2020; McDonough 2020).

At the moment, no strong quantitative limits of BPS and BPF have been fixed yet, neither effective standard analyses nor procedures have been recommended for the quantification in chemicals and leather. In spite of this, low bisphenol products were studied and developed by chemicals manufacturer since the market demand has to be satisfied.

In this work, a fast and non-conventional method for the quantification of BPS and BPF is proposed for the study of low BPS and BPF phenolic and sulphonic syntans. The proposed method is based on chemometric calculations coupled with infrared spectroscopy (IR-S). Chemometrics is defined as "*the discipline that uses mathematical and statistical methods, […] to provide maximum chemical information by analyzing chemical data*" (Otto 2007). Since IR-S is a fingerprint technique, this alternative approach ensures to extract qualitative and quantitative information from spectral data (Brereton 2007). Thanks to this procedure, we developed a rapid, reliable and cheap analytical method, able to supply real time quantification of phenol and bisphenols during the usual productive cycle.

Keywords: syntans, bisphenols, quantification, chemometrics, spectroscopy

1. Introduction

The XX century mass production and use of bisphenol A (BPA) led to a near-universal human exposure because of a deep permeation in the ecosystem (Wazir 2019). A huge number of consumer plastic products contain BPA: food and beverage stuffs, medical stuffs, toys, flooring, furniture, construction materials, textile materials, leather products, paper products, and electronic equipment (McDounough 2021). The implications of this for public safety are noteworthy and BPA has been included in 2017 as substance of very high concern for its reproductive toxicity and for its endocrine disruptor properties (Stokstad 2022).

Bisphenol S (BPS) and bisphenol F (BPF) have found lot of interests in last years as "safer" structural analogue substitutes of BPA. Therefore, BPS and BPF derived products labeled as "BPA free" have been produced and marketed without an effective knowledge on their toxicity. The significant concern about BPS and BPF safety caused in the last decade an exponential increasing of scientific publications in Medical and Health Science peer reviewed journals (research made in https://app.dimensions.ai/; Research filter – *bisphenol s or bisphenol*

f contained in title or abstract; Research Category - *Medical and Health Science*). As structural analogues, BPS and BPF can exert a potential physiological response similar to BPA (Thoene 2020). For this reason, German authorities in 2020 have made a call for evidence to inform their expected REACH restriction proposal covering BPA and related bisphenols. This led to a spread worries in several markets, including the tanning sector, where BPS condensation products are widely used as tanning agent and re-tanning auxiliaries. BPS condensate products are well known among tanners for giving improved light fastness with high grade of whiteness. BPF, instead, typically is a byproduct of phenol-formaldehyde poly-condensation (Covington 2009). In spite of a lack of official regulations and analysis methods, in a context of a general confused scaremongering, the request of low bisphenols (BPs) products and leather arose and grew up in last two years.In a context of an internal R&D project called "B.R.I.D.Ge Syntans", three possible different alternatives were analyzed in order to satisfy this request: the product cutting, the chemical reduction, and the new product development. The principal aim of the project was to give fast replies without altering the chemistry of the final product. In this scenario, the chemical reduction alternative of BPS and BPF was chosen.

Fast responses need fast investigation methods. To overcome the classical approach to the quantification of organic molecules via High Performance Liquid Chromatography (HPLC), "quasi real-time" methods based on Fourier-Transform Infrared Spectroscopy Attenuated Total Reflactance (FTIR-ATR) and Chemometrics were developed starting from the quality control database of routine analysis. Generally, vibrational spectroscopies, such as FTIR-ATR, are fingerprint techniques. They usually provide non- destructive analysis of samples, rapid collection times with no or minimal sample pre-treatment, which reduce the total time and costs of analysis and could support the development of reliable control procedures and screening methods for traceability (Biancolillo 2018). It is already known that quality control operators have to deal rapidly and efficiently with complex issues. The mathematical interpretation of the spectral data can be done with chemometrics, a powerful statistical way to solve complex analytical issues. Chemometrics models, indeed, allows to analyze simultaneously multivariate data set, such as infrared spectra.

The purpose of this work was to provide a fast and reliable method with predictive capacity through a chemometric treatment for the quantification of BPS, BPF and phenol in phenolic and sulfonic syntans starting from known dataset derived from HPLC quantification and IR spectra.

2. Material and Methods

2.1 Samples

103 samples were obtained from different batches production of phenolsulfonic condensations. Because of intellectual property all the synthesis procedures will not be explained in details. In general, single step, double step and sulfonic syntans have been used in this work in a pH range from 3,5 to 4,5 in a concentration ranging from 44% to 50%.

2.2 HPLC measurements

The HPLC system consisted of a Thermo Scientific Vanquish HPLC Series ™ equipped with UV-DAD detector. Chromatographic separation of BPS, BPF and phenol was obtained at 40 °C using a RP C18 2,2 µm 2,1x250 column. The analysis was carried out in a gradient elution reversed-phase mode with a mobile phase consisting in methanol and tetraethylammonium bromide buffer, pH 3,5, adjusted with acetic acid (Baiocchi 1989). BPS and BPF analysis were performed directly by dilution of the liquid sample. Otherwise, in phenol quantification in order to avoid matrix effect, the samples were steam distilled using a Büchi Distillation Unit K-355 and phenol was recovered in a flask as water solution.

2.3 Infrared measurements

FTIR-ATR spectroscopy was performed with a Thermo Scientific Nicolet™ iS™ 5 FTIR Spectrometer equipped with a monolithic diamond crystal. IR spectra were collected in the spectral range between 650 - 1800 cm^{-1} with a resolution of 4 cm^{-1} and 16 scans. The analysis were performed directly on the raw samples without further treatment.

2.4 Multivariate analysis

The raw IR spectra were subjected to Partial Least Squares (PLS) regression using TQ Analyst™ 9.0 software developed by Thermo Scientific.

PLS is one of the standard tools largely used by analytical chemists to make predictions based on a large amount of data collected during chemical processes. In routine analysis, based on the use of PLS regression models, the correlation between the spectral data of samples and their measured related reference values are quantitatively analyzed. The PLS regression algorithm examines the specified spectral regions of the calibration spectra to determine which areas are varying statistically as a function of component concentration. The PLS calibration model is developed in one operation using spectral and concentration information from a set of calibration standards.

Intensity and wavelength are used for our purpose. A PLS regression method is capable of quantifying sample components when the correlation between concentration and absorbance is very complex, for example, when chemical interactions among components cause peaks in the mixture spectrum to shift or broaden or when additional components, whose concentrations are unknown, may be present in the sample mixture. Since PLS is a statistical analysis technique, a large number of standards are needed in order to correlate spectral information with concentration. The spectral variation present in the calibration standards must accurately represent the variation expected in the unknown samples (Geladi 1986).

3. Results and Discussion

The chemistry and synthesis principles of syntans are well known and consolidated. Single step, double step and sulfonic syntans were synthesized similarly as described by Ammen (2015) for second generation, replacement and sulfonic syntans respectively.

In our study, each class of syntans were characterized to have samples with different condensation and sulfonation degrees. All samples were subjected to the usual quality control, which included HPLC analysis and IR spectra. HPLC analysis was used in this work as reference method in the quantification of phenol (LOD 1,82 ppm), BPS (LOD 4,15 ppm) and BPF (LOD 0,50 ppm). HPLC methods were validated using a series of external validation samples with a given concentration of the three analytes.

In Table 1, the concentration range of phenol, BPS and BPF, detected with HPLC, is shown for each class of syntan. As wrote before, the large extent of the analytes concentration detected was related to different synthetic pathways.

Syntan class	phenol (ppm)	BPS (ppm)	BPF (ppm)
Single step	5 – 67	80 – 675	< LOD
Double step	240 – 3580	74 – 5450	208 – 2400
Sulfonic	< LOD	7600 – 16590	< LOD

Table 1 Concentration ranges of phenol, BPS and BPF detected with HPLC in each syntan class

It should be pointed out that all the syntan classes were source of BPS, single and double step were the main source of phenol, whereas BPF was detected only in double step syntans. Starting from these data, because of the complexity of IR spectra of each syntan class, the concentration of each analyte was coupled with specific spectral regions. This allowed to consider simultaneously different spectral factors (wavenumbers) and responses (absorbances), instead of single spectral parameters, such as peak intensity, area or peak ratio, a peculiarity of univariate approaches.

For each analyte a proper quantification method was developed considering defined spectral ranges. In Figure 1 the representative averaged spectra for each syntans class are reported.

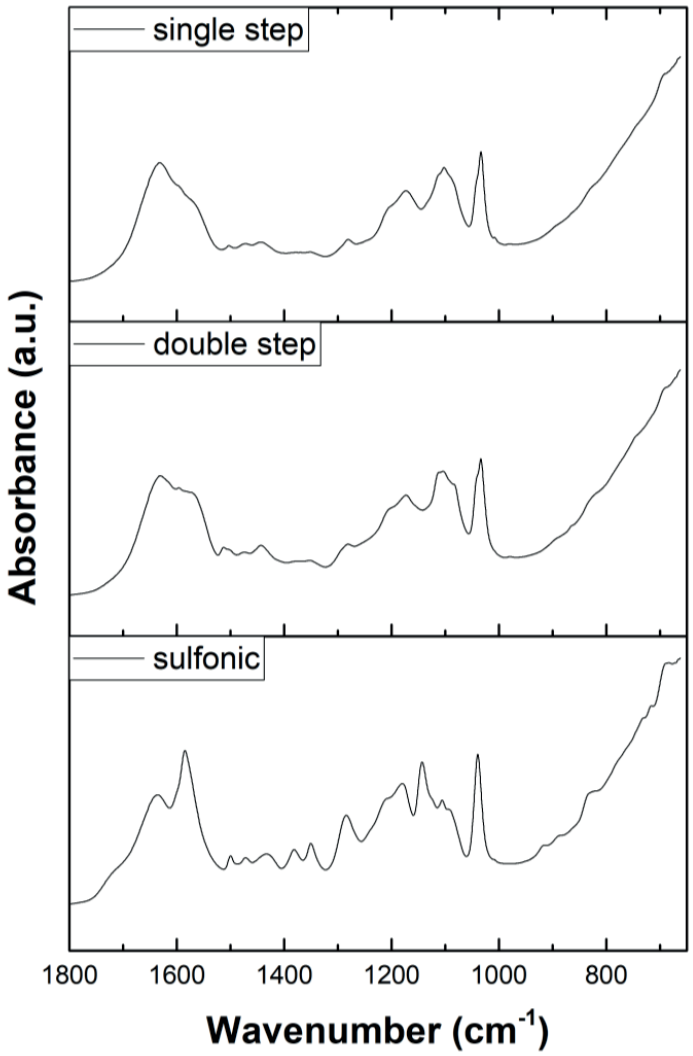


Figure 1: Averaged FTIR-ATR spectra of single step, double step and sulfonic syntans in the spectral region ranging from 650 – 1800 cm^{-1}.

Since syntans have a complex chemical composition and subsequently a complex vibrational pattern, discrete spectral areas have been selected in the region of OH bending and in the region of ring vibration modes at low wavenumbers (Socrates, 2004).

The spectral regions used in the algorithm for BPS, BPF and phenol respectively are reported below:

i) BPS: 650 – 950 cm^{-1}.
ii) BPF: 1450 – 1600 cm^{-1}.
iii) Phenol: 650 – 850 cm^{-1}; 1300 – 1470 cm^{-1} and 1550 – 1600 cm^{-1}.

Training sets for all the methods were built considering at least 50 calibration standards. Figure 2 shows the calibration plots for phenol, BPS and BPF. The black dots in the plots represent the calibration standard selected for the PLS regression.

Each point is defined by two coordinates: the actual concentration measured by HPLC and the concentration calculated by PLS on the basis of the selected spectral variables. Red lines represent linear fit calculated by the model.

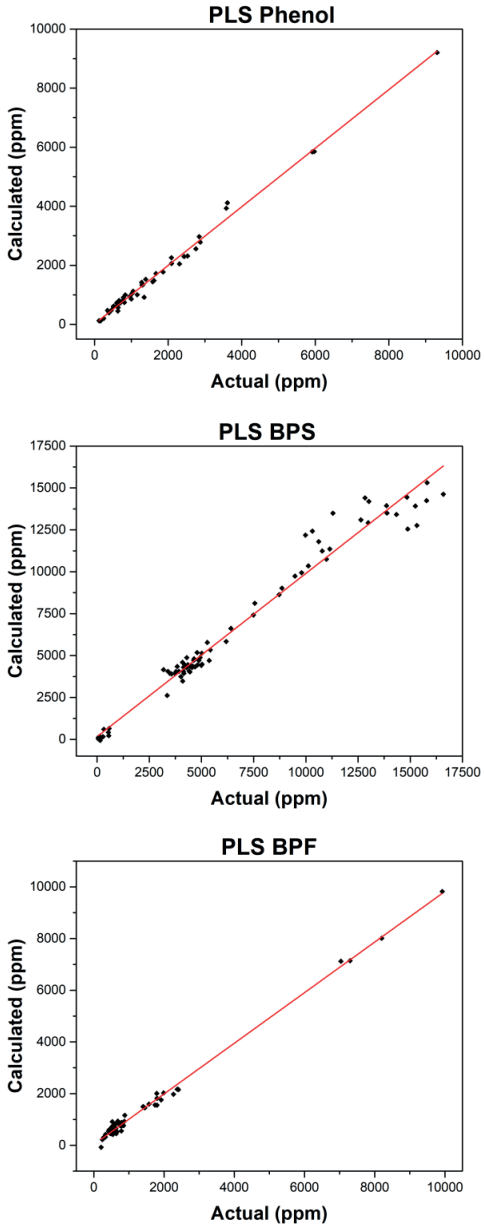


Figure 2: Calibration plots for the quantification models of phenol, BPS and BPF. Actual concentration was plotted against the calculated one.

In Table 2, the principal parameters for the quantification models are shown.

Analyte	R^2	N. of training samples	RMSEC (ppm)	N. of validation samples	RMSEP (ppm)
phenol	0,996	50	157	10	136
BPS	0,988	86	730	17	295
BPF	0,998	57	156	11	143

Table 2: Model parameters for phenol, BPS and BPF quantification.

As reported in Table 2, R^2 represents the correlation coefficient for the plotted values. The closer this value is to

"one" the more linear is the relationship between the calculated and actual values for the selected component. RMSEC (Root Mean Square Error of Calibration) refers to the uncertainty of calibration for the selected component. In order to obtain a numerical estimation about the model predictive capacity, validation samples were selected randomly among real production batches. RMSEP (Root Mean Square Error of Prediction) is a common measurement of the uncertainty for the predictive capacity of a model, comparing predicted values and observed values.

Since R^2 is higher than 0,95, good correlations were observed in each quantification model. It should be noticed that in BPS model despite R^2 is close to one, RMSEC is 730 ppm because of a low correlation between actual and calculated concentration at high values, see Fig.2. The lower correlation is related to a large variability of the calibration samples set. Therefore, the predictive capacity of low BPS content samples would be affected by high uncertainties. As reported in Tab. 1, BPS model was the only one to have all the three syntans classes in a large range of BPS concentrations. A reduction of RMSEC in BPS model probably could be obtained by increasing the number of calibration set samples. On the contrary, the good linearity of phenol and BPF calibration is because the calibration set samples belong to single and double step classes, which have similar spectral pattern. Prediction uncertainty in phenol and BPF quantification is less than 150 ppm.

4. Conclusion

Since BPA analogues were subjected in last two years to a wide concern because of their potential adverse effect to human health, an innovative approach for the quantification of BPS, BPF and phenol was developed.

It is well known that infrared spectroscopy is a fingerprint technique largely used in chemical labs. The coupling of IR spectroscopy and chemometrics guarantees fast, cheap and reliable quantitative analyses without sample treatment and interpretations, useful for a production monitoring. In particular, in this work, three dedicated methods for the quantification of BPS, BPF and phenol in different syntan classes were developed using a PLS regression. The achieved results have shown a good predictive capacity in

the quantification of the three analytes (BPS: RMSEP = 295 ppm; BPF: RMSEP = 143 ppm; phenol RMSEP = 136 ppm). Although far from the predictive capacity of HPLC quantification, the powerful of a multivariate approach in a *quasi*-real time process monitoring was demonstrated, overcoming the classical univariate models.

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