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Optimization of a Dynamic Headspace – Thermal Desorption – Gas Chromatography/Mass Spectrometry procedure for the determination of furfurals in vinegars

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ABSTRACT

The use of a Dynamic Headspace System (DHS) device combined with a Thermal Desorption Unit (TDU) interfaced to a Gas Chromatography/Mass Spectrometry (GC/MS) system is proposed for the determination of furfurals in oenological products. An experimental design protocol has been employed for the optimization of the instrumental settings concerning DHS and TDU extraction and desorption steps. It has been possible to individuate the following optimized conditions: incubation temperature 40 °C, purge volume 800 mL, dry volume 1500 mL, TDU hold time 5 min and incubation time 10 min. The performance of two different SPE sorbents, namely Tenax TA and Tenax GR used for the furfurals trapping, was investigated too.

The developed DHS sampling procedure showed good reproducibility values with a RSD% lower than 10% for all the monitored species. The optimized experimental settings have been used to determine furfurals in several vinegar samples obtained by traditional procedure starting from cooked grape musts, i.e. in Aceto Balsamico Tradizionale di Modena (ABTM). In fact, the control of these species is extremely important for quality and safety issues.

1. Introduction

In recent years, consumers and scientists have become increasingly worried about food components and their effects on human health. The aspects regarding the food safety are closely related to the quality and authenticity of food products, to their production rules as well as to the use of registered denominations such as PDO, protected designation of origin, PGI, protected geographical indication, and STG, traditional speciality guaranteed. In particular, European Union promotes and sustains traditional food as a way to support the quality. Although since 2005 all the state members have adopted the EU 178/2002 rule, in many cases, the chain process controls are still reported as "self-certification". Thus, it often results quite difficult to give detailed information to the consumers about the chemical composition of the product itself owing the absence of suitable technologies and knowledge. For these reasons, it is of utmost relevance to improve the research instruments and methodologies and to develop new analytical procedures in order to provide a detailed characterization of foodstuffs including determination of low concentrations components.

In many cases, the determination of food aromatic profile plays an important role [1] and several analytical approaches for food characterization are based on the analysis of the sample headspace, which allows limited use of solvent and sample preparation. The choice of the more appropriate technique depends on the matrix characteristics, the analytes of interest and the availability of up to date instrumental technologies.

Static headspace (HS), coupled with GC, is a widely used technique to sample analytes in the gas phase [2,3]. This method is based on the partitioning of the analyte between the liquid/solid phase and its headspace and it is particularly suited for light volatiles. One of its main advantages is the simplicity while on the other hand, HS might not always provide adequate detection limits.

Another extraction technique is represented by the solid phase micro-extraction (SPME) [4–7]. The primary advantage of SPME is sensitivity and simplicity of use, while one of the main disadvantages is that SPME does not enable to adsorb high amount of volatiles organic compounds (VOCs) due to the thin coating of adsorbent on the fibre.

The present work focuses on the application of an innovative, automated and successful technique which is capturing the attention of researchers, both in the field of scientific research and quality control: namely, the Dynamic Headspace (DHS) [8–11]. This technique provides samples headspace purging, from solid or

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Table 1a
Experimental plan used for the optimization of the procedure.

TENAX TA						TENAX GR					
Experiments	T_{inc} (°C)	t_{inc} (min)	V_{purge} (mL)	V_{dry} (mL)	TDU_{hold} (min)	Experiments	T_{inc} (°C)	t_{inc} (min)	V_{purge} (mL)	V_{dry} (mL)	TDU_{hold} (min)
N1	40	10	400	1500	5	N21	30	10	400	1500	5
N2	30	20	400	1500	5	N22	40	20	400	1500	5
N3	30	10	800	1500	5	N23	40	10	800	1500	5
N4	40	20	800	1500	5	N24	30	20	800	1500	5
N5	30	10	400	2000	5	N25	40	10	400	2000	5
N6	40	20	400	2000	5	N26	30	20	400	2000	5
N7	40	10	800	2000	5	N27	30	10	800	2000	5
N8	30	20	800	2000	5	N28	40	20	800	2000	5
N9	40	10	400	1500	7	N29	30	10	400	1500	7
N10	30	20	400	1500	7	N30	40	20	400	1500	7
N11	30	10	800	1500	7	N31	40	10	800	1500	7
N12	40	20	800	1500	7	N32	30	20	800	1500	7
N13	30	10	400	2000	7	N33	40	10	400	2000	7
N14	40	20	400	2000	7	N34	30	20	400	2000	7
N15	40	10	800	2000	7	N35	30	10	800	2000	7
N16	30	20	800	2000	7	N36	40	20	800	2000	7
N17	35	15	600	1750	6	N37	35	15	600	1750	6
N18	35	15	600	1750	6	N38	35	15	600	1750	6
N19	35	15	600	1750	6	N39	35	15	600	1750	6
N20	35	15	600	1750	6	N40	35	15	600	1750	6

liquid matrices, by means of an inert gas. Extracted compounds are trapped and concentrated into a tube filled with an appropriate sorbent material; the much higher capacity of the trap, with respect to SPME, permits the extraction of higher quantities of analytes. Due to the wide variety of single and multiple sorbent materials, it is possible to detect compounds with different chemical characteristics. After adsorption, the analytes are thermally desorbed and cryo-focused into the GC injector. The main DHS advantages are low manipulation of sample, low detection limits and high sensitivity.

Aim of this research was the optimization of a Dynamic Headspace sampling procedure coupled with Gas Chromatography/Mass Spectrometry for the extraction and the quantification of furfurals in vinegar samples. In particular, an extensive qualitative and quantitative characterization of furfurals was carried out on Aceto Balsamico Tradizionale di Modena (ABTM) which represents a peculiar and complex food matrix for its chemical/physical features as well as for its making procedure [12,13]. The attention was focused on the quantification of compounds belonging to furfural family, since over the past years, there has been growing interest towards these analytes due to their effects in food as well as in human safety [14–17]. Different works have shown how some of these compounds can be monitored and quantified in foodstuff mainly by using liquid chromatographic techniques (HPLC) [14–19]. The main limits of the HPLC approach, considering the peculiarity of the investigated matrix, i.e. ABTM, lies on the difficulties that may arise by the direct injection of the diluted sample, namely, short life time of the analytical column, high value of detection limit depending on the matrix composition, decrease of detector sensitivity, etc.

The parameters that affect the quantification of the investigated volatile analytes were investigated using a chemometrics approach based on the use of design of experiment technique (DoE). This multivariate approach allows, in a parsimonious way, the simultaneous variation of all the studied experimental factors and their interactions, in order to establish how they influence the procedure; interactions effects are not detectable with the classical univariate, one variable at time, optimization methods [20,21].

2. Experimental design

The optimization of the analytical procedure was performed according to the experiments planned with a full factorial design (FFD), as follows.

Among all the possible variables that might have some effect on the furfurals recoveries, four DHS and one TDU parameters were systematically varied according to the FFD plan: incubation temperature (T_{inc}), incubation time (t_{inc},) purge volume (V_{purge}), dry volume (V_{dry}) and TDU hold time (TDU_{hold}). Essentially, these factors influence different aspects of the VOCs extraction process as well as the desorption procedure. In particular, the incubation parameters are important for the extraction of the volatile compounds while the purge one influences the trapping and the concentration of the analytes on the sorbent material. V_{dry} factor is important to remove the water in order to preserve the chromatographic and MS systems. Finally, the hold time setting on TDU allows maintaining the constant temperature for the desired period to assure the complete desorption of the analytes from the solid sorbent phase.

The two levels, low (−) and high (+), for each of the investigated factors were set on the basis of knowledge of the food matrix properties, as follows: T_{inc}: 30/40 °C and t_{inc}: 10/20 min in order to avoid sample degradation and to allow the reaching of the equilibrium, respectively; V_{purge}: 400/800 mL for optimal trapping of analytes; V_{dry}: 1500/2000 mL for efficiently removing water and TDU_{hold}: 4/6 min.

Two different types of sorbent materials, Tenax TA and Tenax GR, were investigated, hence, the experiments were planned in two different blocks. This ensures that the possible variation due to the differences between the two adsorbent traps does not affect the estimate of the main factor effects.

Thus, a 2^5 full factorial design, with four replicates of the centre point, for each block, was applied. This experimental plan included 40 experiments (32 + 4 + 4) as reported in Table 1a.

The parameters kept fixed and their corresponding values are shown in Table 1b. The experiments were undertaken in random order to prevent confounding from additional source of variability

Table 1b
Constant values used for the optimization of the procedure.

Purge flow (mL min^{-1})	50	End Temp TDU (°C)	300
T_{dry} (°C)	50	Rate TDU (°C min^{-1})	50
Dry flow (mL min^{-1})	100	Initial Temp CIS[a] (°C)	−150
T Transfer DHS (°C)	120	End Temp CIS[a] (°C)	280
T_{purge} (°C)	30	Rate CIS[a] (°C s^{-1})	12
Initial Temp TDU (°C)	50	V_{sample} (mL)	1

[a] CIS: Cooling Injection System.

Table 2
Concentration of the working standards solution.

Compound	Concentration 1 (mg kg^{-1})	Concentration 2 (mg kg^{-1})	Concentration 3 (mg kg^{-1})	Concentration 4 (mg kg^{-1})
furfural (FURF)	0	25	45	95
5-methyl-2-furfural (5-MF)	0	2	5	10
1-(2-furanyl)-ethanone (1,2-EF)	0	1	2	4
5-hydroxymethyl-2-furfural (5-HMF)	0	3000	6000	9000
5-acetoxymethyl-2-furfural (5-AMF)	0	100	250	500

or systematic effects (i.e. day of the run, system performance, etc.). After each experiment, a run of the sorbent material without any analytes extraction, sample blank, was set in order to evaluate the effective desorption of the analytes.

The chromatographic peak areas of five volatile compounds were used as responses: namely, furfural, FURF, 1-(2-furanyl)-ethanone, 1,2-EF, 5-methyl-2-furfural, 5-MF, 5-hydroxymethyl-2-furfural, 5-HMF, and 5-acetoxymethyl-2-furfural, 5-AMF.

A principal component analysis (PCA) [22] was performed on the obtained data, in order to highlight relationships among the responses and the experimental runs. Finally, the experiments were evaluated using multiple linear regression (MLR) to obtain a response model for each species.

PCA was carried out by using PLS-Toolbox© (version 5.2 distributed by Eigenvector Research, Inc., 3905 West Eaglerock Drive, Wenatchee, WA 98801). Designs of experiments were planned with Modde (Umetrics). The experiments were evaluated using multiple linear regression (MLR) routines written by Dr. Riccardo Leardi in MATLAB© environment.

3. Experimental

3.1. Sampling

3.1.1. Food matrix used for DHS optimization procedure (sample test)

In order to evaluate the possible influence of the matrix during the VOCs' determination, an Aceto Balsamico Tradizionale di Modena sample (ABTM) ($d = 1.345 \pm 0.001$ g mL^{-1}), named sample test, was used to perform a full factorial design.

3.1.2. Artificial matrix and standards used for analytes quantification

In the present case, given the lack of appropriate certified reference materials, a solution with approximately the same physical/chemical characteristics of ABTM, named artificial matrix, was used to determine the concentration of furfurals in ABTM. It is worth noticing that a solution miming ABTM, i.e. containing sugars, organic acids, water, glycerine and dissolved salts, is characterized by: (a) density values far higher than water one, (b) the presence of a "pseudo glassy" molecular structure which is mainly related to the glucose/fructose ratio and the content of polymeric species formed during the production process. Obviously, these peculiarities of the sample matrix contribute to alter the viscosity/rheology of the product [23]. Hence, more accurate is the chemical composition of the artificial matrix used for miming ABTM, more reproducible will be the chemical–physical behaviour of the sample.

On the basis of this consideration, the artificial matrix was made as follows: 33% glucose, 33% fructose, 4% malic acid, 3% tartaric acid, 1.5% glycerol, 0.3% KCl, 0.3% $MgCl_2$, 0.2% $CaCl_2$ and 0.1% NaCl in water. This solution has a density of $d = 1.3749$ g mL^{-1}.

For the determination of calibration curves, different amounts of furfurals were added to the artificial matrix as reported in Table 2.

3.1.3. ABTM samples

The procedure optimized by DoE was used for the determination of the aromatic profile of 29 ABTM samples. Table A1 (Supporting Material) reports density, acidity and refractive index for each analysed sample. Data represent the average of three independent replicates acquired for each vinegar sample and the details concerning these determinations were previously reported [24].

On the basis of previous studies [25], 0.25 g of NaCl were added to each ABTM prior to the DHS–TDU–GC/MS analysis, in order to keep constant the ionic strength of the ABTM samples and to prevent the possible matrix effects on the vapour pressure of the monitored moieties. Each investigated sample was at least double analysed.

3.2. Chemicals

Purified water was obtained with a Millipore Milli Q Plus system. Standards of furfural (FURF), 5-methyl-2-furfural (5-MF), 5-acetoxymethyl-2-furfural (5-AMF), 1-(2-furanyl)-ethanone (1,2-EF) and 5-hydroxymethyl-2-furfural (5-HMF), with purity greater than 99%, were supplied by Sigma–Aldrich. Tartaric and malic acid, with 99% purity grade, and fructose and glucose, with 99.5% purity grade, were purchased from J.T. Baker. NaCl, $MgCl_2$, KCl, $CaCl_2$ were provided by Carlo Erba (Milan, Italy), with purity greater than 99%.

3.3. Apparatus

Furfurals extraction was performed by means of a Dynamic Headspace unit mounted on a MultiPurpose MPS2 XL autosampler. Two kind of sorbent material were tested: Tenax TA and Tenax GR. Thermal desorption and cryofocusing of the volatile compounds were performed by means of a Thermo Desorption Unit (TDU) and a Cooling Injection System (CIS4), respectively. All the instrumental equipments and the sorbent tubes were supplied by Gerstel.

Analytes separation, identification and quantification were carried out by means of GC technique using a Varian CPSil 8CB (60 m × 0.25 mm × 1 μm) low bleed/MS column mounted on an Agilent 6890 GC couplet with an Agilent 5973N MS analyzer.

GC separation was performed with the following program: starting oven temperature 40 °C, with 2 min hold time, then to 150 °C at 5 °C min^{-1}, followed by subsequent temperature increase to 280 °C at 10 °C min^{-1} and 10 min of final hold time. The temperature of the MS transfer line was set at 290 °C. The carrier gas was helium. The chromatographic separation was performed in constant flow mode at 1 mL min^{-1}.

The chromatograms were collected in full scan mode. The electron impact, EI, spectra were recorded from 25 to 450 m/z, with 70 eV ionization energy and a sampling frequency rate of 3 scan s^{-1}.

Analytes were firstly identified by NIST and Wiley library and then confirmed by means of standards. In order to minimise background interferences, peaks areas were always calculated by extracting the primary ion contribution from the Total Ion Current (TIC).

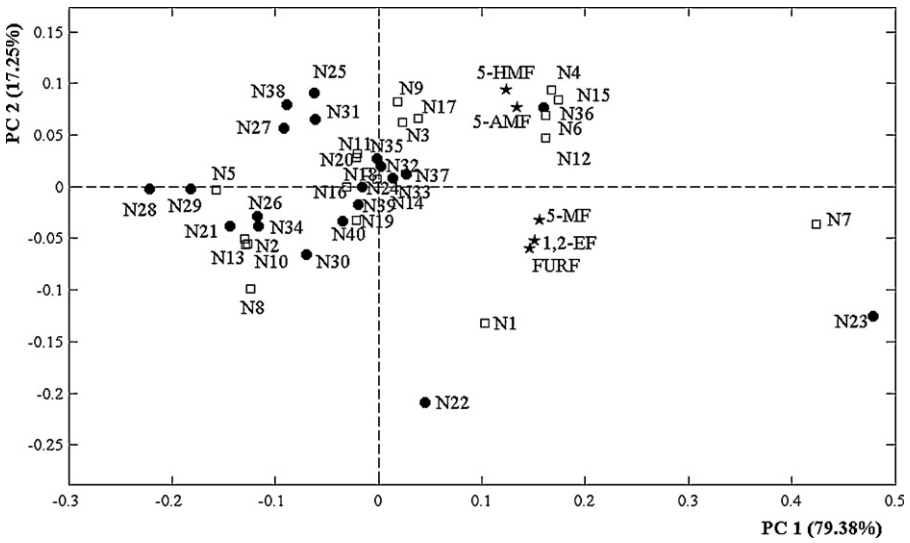

Fig. 1. Biplot of PC1 vs. PC2 of the results obtained with DoE experimental plan. Experiments performed with Tenax TA and Tenax GR are labelled with '□' and '●' respectively. The stars indicate the variables, i.e. the responses (labelled by their names). Each experiment is labelled by its number preceded by "N".

3.4. Analytical procedure

The extraction of the analytes was carried out using the optimized conditions obtained by means of the experimental design procedure. In particular, 1 mL of sample was placed into a 20 mL glass vial, added of 0.25 g NaCl as ionic strength modifier, thermostated at 40 °C and agitated for an incubation time of 10 min. Afterwards, the sample headspace was purged with a N_2 flow of 50 mL min^{-1} for a total purge volume of 800 mL and the analytes collected on the SPE tube set at 30 °C. The temperature of the DHS needles was kept constant at 120 °C allowing VOCs to be driven from the sample vial directly into the SPE tube. In order to reduce the amount of aqueous vapour sampled, the tube was dried at 50 °C with a N_2 flow of 100 mL min^{-1} for a total volume of 1500 mL. After the dry purge step, the tube was automatically moved to the TDU unit for the desorption of VOCs in splitless conditions with the following heating program: from 50 to 280 °C at 50 °C min^{-1} with a final hold time of 5 min. Analytes were then cryofocused in the CIS4 injector cooled at −150 °C by means of liquid N_2 and successively desorbed from −150 to 280 °C at 12 °C s^{-1} with a final hold time of 1 min. The temperature of the transfer line between the TDU and the CIS4 was kept constant at 290 °C. VOCs were directly transferred into the column with a split ratio of 1:20.

4. Results and discussion

4.1. Full factorial design

For the optimization procedure, all the DHS experiments were carried out on the sample test, according to the scheduled operations reported in Table 1.

The centre points and their replicates, for each block, were added in order to check for the presence of non-linear relationship between the variables and the responses, as well as to investigate on the repeatability, defined as an internal short term precision (intra-day) and reproducibility, defined as a long term precision of the measurements performed by different users, instrumentation, etc. (inter-day), of the measurements. Furthermore, the peaks areas relatively to the five investigated species have been considered as analytical responses.

In order to evaluate the repeatability of the proposed method, three replicates of the sample test were performed in the same day (one at the beginning, one at the middle and one at the end of the experimental session, respectively) using the experimental conditions of the centre point for both the tested sorbent phases. On the other side, reproducibility was evaluated by considering the responses obtained for the four experimental runs of the centre points in four different days. In both cases, considering the peaks areas of the investigated species, relative standard deviation has been computed, namely RSD_{intra} and RSD_{inter} for repeatability and reproducibility studies, respectively. In particular, RSD_{intra} ranges between 4 and 6% for both the sorbent phases, while RSD_{inter} is lower than 10% in the case of Tenax TA, and ranges from 10% to 28% for the Tenax GR ones.

In order to visualize and extract useful information about the relationships among the DoE experimental runs and the analytical responses, the response data were arranged in a data matrix constitutes by 40 rows (experimental runs) × 5 columns (peak area of the 5 investigated analytes), which was analysed by PCA (after columns autoscaling pretreatment). The model was built with 2 PCs (explained variance in fit 96.63%). The biplot of PC1 versus PC2 is reported in Fig. 1.

As can be seen in the figure, the replicates for each sorbent phase (N17–N20 relatively to Tenax TA and N37–N40 for Tenax GR) are well grouped and close to the origin of the axis, indicating good repeatability and reproducibility of the instrumental procedure.

As far as the trapping capability of the two sorbent phases is concerned, with the exception of experiments N7 and N23, the two PCs do not show significant differences. In fact, there is a wide overlapping among the experiments run with Tenax TA and Tenax GR.

N7 (carried out with Tenax TA) and N23 (with Tenax GR) experiments show the highest positive score values on PC1. These two runs differ from each other for the sorbent phase used and for V_{dry} setting condition (high level for N7 and low for N23). Since the loadings of all the variables on PC1 are directly correlated and get positive values, the conditions planned in N7 and N23 runs seem to correspond to better performance with respect to the other runs settings.

Finally, the furfural species are mainly differentiated on PC2. In fact, along this component, it is possible to distinguish two groups, namely FURF, 5-MF, 1,2-EF and 5-HMF and 5-AMF, inversely correlated. The species belonging to the same group probably show a similar 'adsorbent' behaviour as a function of the different instrumental conditions.

Table 3
Significant coefficients included in the response model.

Coefficients	Terms of the model	Peak area values
b_1 ***	T_{inc}	2.75×10^7
b_2 *	V_{purge}	1.39×10^7
b_8 *	T_{inc} TDU_{hold}	-1.65×10^7
b_{12} *	V_{purge} t_{inc}	-1.45×10^7
b_{13} *	V_{dry} TDU_{hold}	1.66×10^7
b_{15} *	TDU_{hold} t_{inc}	1.49×10^7
b_{18} *	T_{inc} V_{purge} t_{inc}	-1.27×10^7
b_{21} *	T_{inc} TDU_{hold} t_{inc}	1.43×10^7
b_{24} *	V_{purge} TDU_{hold} t_{inc}	1.67×10^7

* $p < 0.05$ denote the statistical significance of the coefficients.
*** $p < 0.001$ denote the statistical significance of the coefficients.

Both PCA and RSD values of peaks area pointed out a good precision as well as a comparable trapping capability for the two sorbent phases. Notwithstanding, it has been decided to use Tenax TA sorbent phase for the sampling of furfural species, since its reproducibility is higher with respect to Tenax GR.

After this preliminary exploratory analysis, the experiments were evaluated using MLR to obtain a predictive model for each response, i.e. peak area of a given volatile compound. The applied experimental design allows the estimation of the coefficients (b) of the following postulated model:

$$y = b_0 + \sum_{i=1}^{5} b_i x_i + \sum_{i=1}^{5}\sum_{j=1}^{5} b_{ij} x_i x_j + \sum_{i=1}^{5}\sum_{j=1}^{5}\sum_{k=1}^{5} b_{ijk} x_i x_j x_k$$

where b_0 is the constant term, b_i are the coefficients that account for the linear effects, b_{ij} (with $i \neq j$) and b_{ijk} (with $i \neq j \neq k$) are the coefficients for the second and third order interaction terms, respectively.

For the sake of brevity, only the model obtained for FURF is discussed. The values of the significant coefficients have been reported in Table 3.

As far as the main terms are concerned, both T_{inc} and V_{purge} coefficients are positive and indicate that the best sorption condition for FURF is obtained when high values of these factors are set. V_{dry}, TDU_{hold} and t_{inc} have no significant effect as main factors, but they are of some relevance when considering second and third order interactions.

Fig. 2, as an example, shows the response surface plot for FURF obtained by plotting T_{inc}, vs. TDU_{hold}, estimated by the MLR model ($R^2_{adjusted}$: 67%, where $R^2_{adjusted}$ is the explained variance corrected by the degrees of freedom) built by only considering the statistically significant terms, when fixing the V_{purge} at high level and V_{dry} and t_{inc} at the low ones. The maximum of the surface corresponds to high level for T_{inc} and low level for TDU_{hold}. This interaction indicates that high incubation temperature produces the extraction of a larger amount of FURF when a low TDU hold time is used.

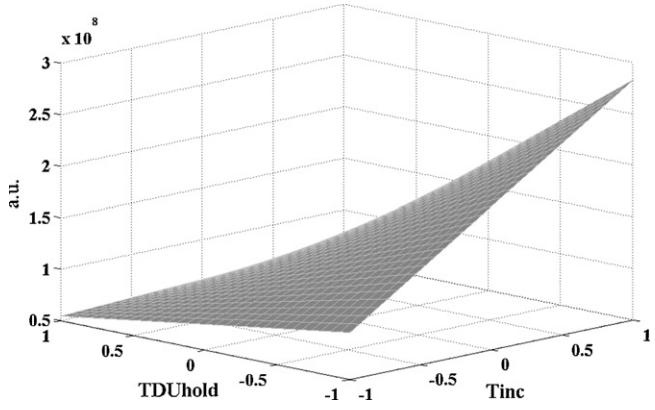

Fig. 2. Estimated response surface for furfural species.

Table 4
Explained variance ($\%R^2_{adjusted}$) by the different models and factor levels corresponding to best response for each investigated species. Last row reports the best compromise settings.

	$\%R^2_{adjusted}$	T_{inc}	V_{purge}	V_{dry}	TDU_{hold}	t_{inc}
FURF	63	+	+	–	–	–
1,2-EF	58	+	+	–	–	–
5-MF	52	+	+	–	–	–
5-HMF	49	+	+	+	+	–
5-AMF	76	+	+	–	–	–
Chosen levels		+	+	–	–	–

In Table 4, are summarized the results obtained by considering the MLR models for each of the investigated furfurals species. Furthermore, for each response, are reported the values of the explained variances corrected by the degrees of freedom, $\%R^2_{adjusted}$, obtained by fitting MLR models including all the terms.

The symbols (+) and (–) correspond to the high and low level for each factor, respectively. As can be seen, high values of incubation temperature and purge volume positively increase the sampling of all the analytes, while the opposite holds for TDU hold and incubation times. Dry volume resulted significant (positive sign) for all the furfurals with the exception of 5-HMF species. These experimental conditions are in accordance with PCA results, indeed.

In summary, after the evaluation of PCA results and MLR coefficients, the best conditions for the extraction and trapping of furfurals were recognized as the following: high level for T_{inc} (40 °C), high level for V_{purge} (800 mL), low level for V_{dry} (1500 mL), low level for TDU_{hold} (5 min), low level for t_{inc} (10 min) and Tenax TA as stationary sorbent phase.

4.2. Furfurals quantification in ABTM samples

Generally, there is a huge difference between the experimental approaches adopted in order to obtain a qualitative (what constitutes) or a quantitative (amount of major, minor, trace and ultra trace constituents) characterization of a sample.

In many cases, determination of accuracy, precision and recovery of analytical methods, i.e. validation procedures, is difficult to perform owing to, for instance, the lack of appropriate standards, certified reference materials (CRM) or official analytical methods. In these situations, alternative strategies have to be adopted, which, of course, may introduce some approximations whose relevance is peculiar to the investigated system, in order to deal with the so called "matrix effect". In particular, to calculate the concentration of chemical species the following analytical approaches can be proposed: (i) use of standard solutions; (ii) use of the standard addition method, SAM; (iii) use of standards in an artificial matrix; and (iv) use of the internal standard technique.

In this study, several approaches were tried for the quantification of the investigated species in the vinegar samples.

Initially, owing to the peculiarities of the sample matrix, the method of standard addition was tested. Standard solutions of FURF, 1,2-EF, 5-MF, 5-HMF and 5-AMF (Table 2) were separately added to an ABTM sample and analysed with the DHS–TDU–GC/MS optimized procedure.

As FURF, 1,2-EF and 5-MF are concerned, the calibration curves resulted to be linear over the examined concentration range and in Table 5, the m/z data of the selected ions, the correlation coefficients and the equations of their calibration curves and their limit of detection (LOD) were reported. The concentrations determination was performed by means of $y = mx$ regression equation since the intercept term was statistically equal to zero for all the investigated species.

Table 5
m/z, regression coefficient and slope of the calibration curves, and limit of detection for the investigated volatile compounds.

	m/z	Correlation coefficient (R^2)	Slope ± SD	LOD ($\mu g\,kg^{-1}$)
FURF	96	0.9988	1,230,890 ± 23,853	500
1,2-EF	95	0.9927	2,520,952 ± 152,901	60
5-MF	110	0.9959	1,392,662 ± 64,063	100

With regard to 5-HMF and 5-AMF, the calibration curves did not result to be linear over the examined concentration range and this approach did not give useful results owing also to the scarce reproducibility and repeatability. This is probably due to (i) the presence of interfering equilibria among the added chemicals in solution and the sample matrix and (ii) the low vapour pressures of these species (high water affinity). Furthermore, the behaviour for 5-HMF has been previously highlighted by different authors [7] that achieved quite low quantification recoveries for spiked solution by using a different analytical technique.

Due to the lack of certified reference material, an artificial matrix with a chemical composition similar to the ABTM was used. The artificial matrix was then added with known amounts of furfurals to obtain suitable calibration curves, as previously reported (Section 3.1.2). Successively, an aliquot of 1 mL of each solution was analysed by applying the optimized analytical procedure.

The FURF, 1,2-EF and 5-MF calibration curves resulted to be linear over the examined range (Table 2); moreover, the comparison, based on Student's *t*-test (at confidence level of $p \leq 0.05$), between the respective calibration curves obtained with real and artificial matrices, has highlighted that the artificial matrix seems to perfectly match the real sample.

On the other hand, as regards 5-HMF and 5-AMF, also by this experimental approach, it has not been possible to obtain or verify any linear trend between the added amount of standard and the instrumental response in the considered concentration range. In order to verify if the simultaneous presence of various chemical species could interfere with the extraction and sampling of 5-HMF and 5-AMF, the artificial matrix was separately added with each single standard to obtain a calibration curve for every investigated compound.

Also with this approach it has not been possible to obtain linearity and satisfactory repeatability, reproducibility and accuracy, providing no improvements compared to previous efforts.

This behaviour is probably due to the low vapour pressure of these species on one hand and to the difficulties in making a "solutions" of these species in the investigated matrix: ABTM or artificial matrix as well. Hence only FURF, 1,2-EF and 5-MF were quantified in the ABTM samples.

Fig. 3 shows a typical chromatogram obtained from the analysis of a vinegar sample with the optimized experimental conditions. The extraction procedure, performed with Tenax TA, allows the sampling of a huge number of VOCs.

In addition to the molecules used for the optimization of the procedure, it is possible to detect several other furfurals, for instance 2-furoic acid, for which the quantification is still in progress.

The range of concentration of the three investigated analytes, obtained for 29 different ABTM samples, are reported in Table 6.

For the same samples the FURF content was also evaluated by means of HPLC technique [17]. Aiming at validating the

Table 6
Concentration ranges and averaged concentration values with respective standard deviations, for FUR, 1,2-EF and 5-MF evaluated in ABTM samples.

Parameter	FURF	1,2-EF	5-MF
Max conc. value/mg kg^{-1}	46.9	1.42	8.37
Min conc. value/mg kg^{-1}	24.0	0.37	1.28
Mean conc. value/mg kg^{-1}	33.9 ± 7.2	0.54 ± 0.20	2.98 ± 1.37

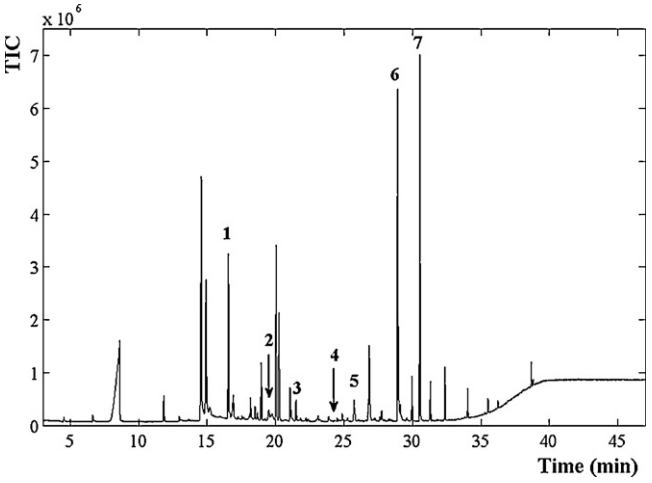

Fig. 3. Total ion chromatogram obtained for a vinegar sample by means of DHS–TDU. (1) furfural; (2) 1-(2-furanyl)-ethanone; (3) 5-methyl-furfural; (4) furoic acid; (5) methyl-2-furoate; (6) 5-hydroxymethyl-2-furfural; (7) 5-acetoxymethyl-2-furfural.

experimental approach, the accuracy of the proposed method has been assessed comparing the results obtained with both HPLC and DHS approaches. In particular, a Student's *t*-test (at confidence level of $p \leq 0.05$) has been performed assessing that there is not statistically significant differences between the furfural values estimated by DHS and HPLC, respectively.

Fig. 4 reports a graphical comparison of the furfural amount obtained with both HPLC and DHS techniques.

As it can be seen, both the techniques led to similar furfural values except for samples number 4, 7 and 18. For these latter samples, the difference, with respect to the HPLC value, is higher than 10% of the measured values. At the moment, the reasons for these differences between the HPLC and DHS data are not clear since the bulk composition of these three samples should be within the

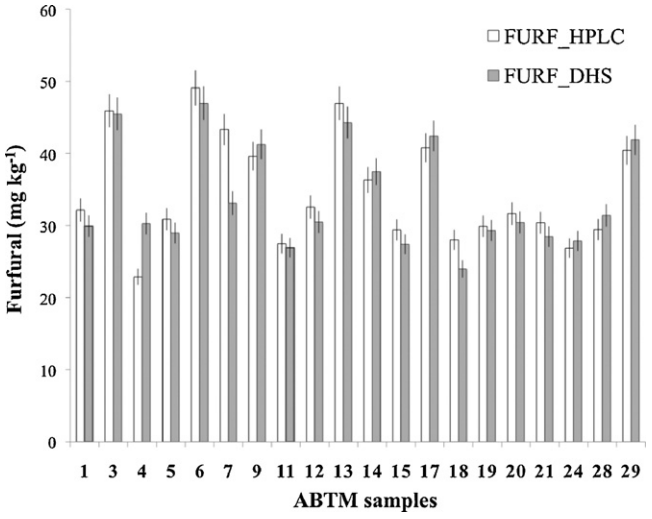

Fig. 4. Furfural concentrations obtained with HPLC (white bars) and DHS (grey ones) techniques. The upper error bars represent the standard deviation evaluated from replicates samples.

composition range of all the investigated samples. On the other hand, the vapour pressure of the sampled analytes is highly dependent on the matrix composition and this fact can alter the recovery of each species. The most powerful strategy to overcome the effects due to the matrix is, probably, the use of isotopic species such as deuterated furfural ($C_5D_4O_2$). Although this approach is feasible from a technical point of view, the analytical costs are "prohibitive". Therefore, to obtain more information about HPLC–DHS differences, further chemical characterization of the samples is needed, with particular attention to the chemical/physical properties that mainly influence the colligative properties of these real systems.

As far as the 1,2-EF and 5-MF are concerned, due to the lack of official methods as well as to the limits in the use of the standard addition method (i.e. presence of systematic errors) [13,19,26], it has not been possible to perform any analytical tests in order to evaluate the recovery. Notwithstanding, the results obtained for all the investigated species by the present approach are quite in accordance with that ones obtained on homologous matrices by means of others analytical techniques [7,27].

5. Conclusions

DHS–TDU–GC/MS technique was successfully applied to the determination of furfurals in oenological products, namely the Aceto Balsamico Tradizionale di Modena. This methodology resulted to be quite simple, fast and, thanks to the high automation level, it is possible to obtain good reproducibility and repeatability of the data. The design of experiment methodology allowed optimization of the extraction of the analytes of interest: the evaluations of the experiments by PCA results, and of the model postulated with MLR for all the responses, resulted to be in agreement, highlighting the robustness of the optimized conditions. Moreover, the quantification of the furfurals resulted to be strictly dependent on the vapour pressure of the analytes, in fact it was possible to quantify only furfurals with a relatively high vapour pressure, such as FURF, 1,2-EF and 5-MF. Furthermore, there was a good agreement between the values obtained with both HPLC and DHS–TDU–GC/MS techniques, even if some problems were highlighted in the quantification of the investigated furfurals for few ABTM samples. From these results, it is worth to note that some chemical and physical features of the samples (i.e. density, viscosity, acidity, etc.) should be taken into account in the optimization of the procedure and for a deepest knowledge of the influence of these factors on the investigated responses. Only in this way, it will be possible to render this analytical procedure generally applicable to different kind of vinegars and in general, on oenological products or other food.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2011.04.018.

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