Journal of Chromatography A, 1218 (2011) 3317–3325

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Qualitative and quantitative analysis of pyrolysis oil by gas chromatography with flame ionization detection and comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry

Themistoklis Sfetsas[a,*], Chrysa Michailof[a], Angelos Lappas[a], Qiangyi Li[b], Brian Kneale[b]

[a] Centre for Research & Technology Hellas, Chemical Process Engineering Research Institute, 6th km Charilaou–Thermi Road, P.O. Box 361, 57001 Thessaloniki, Greece
[b] BP Products North America Inc., 150 Warrenville Road, Naperville, IL 60563-8473, USA

ARTICLE INFO

Article history:
Available online 13 October 2010

Keywords:
GC-FID
GC × GC–TOFMS
Pyrolysis oil
Classification
Quantification
Weighted linear regression

ABSTRACT

Pyrolysis oils have attracted a lot of interest, as they are liquid energy carriers and general sources of chemicals. In this work, gas chromatography with flame ionization detector (GC-FID) and two-dimensional gas chromatography with time-of-flight mass spectrometry (GC × GC–TOFMS) techniques were used to provide both qualitative and quantitative results of the analysis of three different pyrolysis oils. The chromatographic methods and parameters were optimized and solvent choice and separation restrictions are discussed. Pyrolysis oil samples were diluted in suitable organic solvent and were analyzed by GC × GC–TOFMS. An average of 300 compounds were detected and identified in all three samples using the ChromaToF (Leco) software. The deconvoluted spectra were compared with the NIST software library for correct matching. Group type classification was performed by use of the ChromaToF software. The quantification of 11 selected compounds was performed by means of a multiple-point external calibration curve. Afterwards, the pyrolysis oils were extracted with water, and the aqueous phase was analyzed both by GC-FID and, after proper change of solvent, by GC × GC–TOFMS. As previously, the selected compounds were quantified by both techniques, by means of multiple point external calibration curves. The parameters of the calibration curves were calculated by weighted linear regression analysis. The limit of detection, limit of quantitation and linearity range for each standard compound with each method are presented. The potency of GC × GC–TOFMS for an efficient mapping of the pyrolysis oil is undisputable, and the possibility of using it for quantification as well has been demonstrated. On the other hand, the GC-FID analysis provides reliable results that allow for a rapid screening of the pyrolysis oil. To the best of our knowledge, very few papers have been reported with quantification attempts on pyrolysis oil samples using GC × GC–TOFMS most of which make use of the internal standard method. This work provides the ground for further analysis of pyrolysis oils of diverse sources for a rational design of both their production and utilization process.

1. Introduction

The environmental problems associated with the extended use of fossil fuels are well known and established, and in addition to their forthcoming shortage, scientific interest has turned towards the development of technologies exploiting renewable energy sources. Such an alternative source is biomass, which as a term is used to define any type of organic residue or certain types of energy crops that may be used as a renewable energy source. Biomass is a complex material, mainly composed of hemicellulose, cellulose and lignin in addition to extractives (tannins, fatty acids, resins) and inorganic salts [1]. With an increasing global population, more and more biomass residues are being generated, due to higher demands for food and shelter. Development of advanced state-of-the-art commercial technologies such as fast pyrolysis will help treating the waste generated, thereby decreasing environmental pollution, and permitting conversion of agricultural biomass into useful bio-products [2]. Fast pyrolysis is a process that produces gas, solid and liquid products. The liquid product of fast pyrolysis is commonly called bio-oil or pyrolysis oil. Bio-oils find applications as sources of chemicals, as fuels, mainly in mixtures or emulsions with fossil fuels [3,4], while their use as fungicides or wood preservatives, has also been proposed [5,6]. They can also be upgraded through hydrodeoxygenation or catalytic cracking, so as to reduce their viscosity or oxygen content in order for them to be incorporated into existing technologies.

Bio-oil is a mixture of various chemical compounds including carboxylic acids, guaiacols, syringols, phenols, etc., as a result of the

* Corresponding author. Tel.: +30 2310 498305; fax: +30 2310 498380.
E-mail address: sfetsas@cperi.certh.gr (T. Sfetsas).

0021-9673/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2010.10.034

rapid depolymerization and the chemical fragmentation of lignin, cellulose and hemicellulose during the fast pyrolysis. The chemical composition of bio-oils is determined by the nature of the biomass from which they originate and the pyrolysis conditions employed [7,8]. Catalytic biomass pyrolysis has been extensively researched for the production of bio-oil and upgraded biofuels in a one-step procedure [9,10]. The bio-oil resulting from such a process usually consists of two phases: a lower mainly organic phase containing oligomeric lignin molecules and other compounds with high molecular weight, and an upper aqueous phase, which is rich in low molecular weight compounds with added value, such as carboxylic acids, phenols, guaiacols, syringols, etc. The chemical composition of this aqueous phase renders it suitable as feedstock in a steam reforming process for the production of hydrogen [11] or after further fractionation for the extraction of specific chemicals for other applications, such as syringols or guaiacols that maybe used as flavorings [7], etc. Such an aqueous phase is also obtained from single-phase bio-oils through water extraction. In any case it is important to know the content of the chemicals of interest in each phase, for a rational design of any process.

The bio-oil's complex nature renders essential the use of high resolution chromatographic techniques. Both HPLC [12,13] and GC [14–16] have been employed, since the bio-oil contains both volatile and non-volatile compounds, with mass detection being the most efficient medium for clarifying the identity of the numerous unknown compounds. Among the available chromatographic techniques, GC × GC–TOFMS is very popular for the analysis of bio-oil due to its very high resolution power.

Comprehensive GC × GC–TOFMS was applied to pyrolytic oils in order to demonstrate the advantages of GC × GC. The results of this study indicate that more than 70% of total chromatogram peaks could be identified with GC × GC but only 47%, at best, with conventional GC. The increase in the number of identified products is due to increased number of separations [17]. Comprehensive GC × GC-FID and GC–MS analysis of pyrolysis oil and hydrodeoxygenated (HDO) oils was also reported by Marsman et al. [18]. In this work identification was performed by GC–MS and quantification of group type classified compounds by GC × GC-FID. This paper also describes the first approach to classify the various components existing in pyrolysis oils. One year later in 2008 Marsman et al. applied GC × GC–TOFMS in pyrolysis and HDO comparing different catalysts for hydrotreatment and also classifying groups of compounds, for both flash pyrolysis oil and HDO oil [19].

For the purpose of analyzing lignin, a special offline pyrolysis rig was designed by Windt et al. In this case the obtained pyrolysis products were classified into three groups: coke, liquid and gas phase (consisting mainly of VOCs and permanent gases). The liquid fraction was analyzed by GC–MS/FID and GC × GC–TOFMS/FID [20]. A production method of pyrolytic liquids with the application of induction-heating, has been reported for sewage sludge from food processing factories in an externally heated fixed-bed reactor by Tsai et al. [21]. The latter samples from the pyrolysis of industrial sewage sludge using induction-heating technique, were analyzed by GC–MS by Tsai et al. [22]. Through py-GC–MS Bayerbach and Meier characterized pyrolytic lignins in the water insoluble fraction [23]. Dalluge et al. published a review of several applications of GC × GC and group-type analysis devoted to the principle, advantages, and main characteristics such as modulation, column combinations, detector requirements and data processing, of the technique [24]. A more recent review summarizes the literature on comprehensive two-dimensional gas chromatography (GC × GC), with emphasis on application-oriented studies published in the period 2004–2006 and the high potential of GC × GC combined with time-of-flight mass spectrometry [25]. Composition analysis, by combination of several chromatographic techniques, following fractionation of biomass-based flash pyrolysis oils had also been published in the past by Desbene et al. [26].

Apart from the undisputable separation capacity of GC × GC–TOFMS for the qualitative analysis of bio-oils, it may also be used for their quantitative analysis. Special consideration in many chemometric techniques has proven to be necessary. Very comprehensive is the review published by Amador-Munoz and Marriott [27] who investigate the role of quantitative analysis through calculation of the peak areas and peak area ratios of selected series of modulated peaks in GC × GC. In their work, isotopically labeled reference compounds for polycyclic aromatic hydrocarbon (PAH) analysis were used to develop the quantitative metric approach. de Godoy et al. proposed in their work a methodology for the quantification of kerosene in gasoline through N-way multivariate analysis by using a GC × GC-FID system [28]. However, for a quantitative analysis to be performed by such a technique, along with the proper statistical manipulation of the data, special chemometric techniques need to be applied and therefore a proper integrated software is needed, as indicated by the review of Pierce et al. [29] who summarize the trends of the recent past and the software developments, as a sector of emerging and promising growth.

In the light of the elevated cost of such a technique, and the careful standardization needed for reliable quantitative results, the use of more conventional chromatographic techniques such as GC-FID should not be overlooked, since it may provide an efficient and cost effective way for the primary analysis of various bio-oils. In any case, this technique does not allow the full mapping of bio-oil, however it is suitable for the qualitative and quantitative determination of certain target compounds that are expected to exist in the bio-oil, and could determine the best way for its utilization or indicate how pyrolysis conditions could be changed in order to obtain a bio-oil of desired synthesis.

This paper addresses the issue of qualitative and quantitative analysis of bio-oils by comparing the quantitative results for ten selected compounds determined by both GC × GC–TOFMS and GC-FID. The quantitative analysis was performed by external calibration and the results were fitted to the weighted linear regression model. Calibration curves, linearity range, limits of detection and quantitation are presented for each compound, thereby providing a direct comparison of the chromatographic techniques. To the best of our knowledge, this is one of the few publications [30] addressing the issue of detailed quantification of bio-oils.

2. Experimental

2.1. Reagents and standards

All standard compounds and reagents used were of GC-grade and were purchased by Sigma–Aldrich. The standard solutions prepared for the GC-FID measurements consisted of ten compounds: cyclopentanone, hydroxypropanone, 2-cyclopenten-1-one, acetic acid, 2-furaldehyde, 3-methyl-2-cyclopenten-1-one, 2-acetyl-5-methylfuran, 2(5H)furanone, guaiacol and syringol. An aqueous stock solution was prepared by accurate weighting of the above compounds. The six standard solutions, with concentrations of each compound ranging from 15 to 640 $\mu g\,mL^{-1}$, were prepared from the stock solution after proper dilution. The samples were injected in five replicates. The standard solutions were kept in the refrigerator until use.

For the case of GC × GC–TOFMS, a stock solution with 11 substances was prepared in acetone and a second stock solution with 10 substances was prepared in dichloromethane. The standard compounds used were the ones mentioned above, with the addition of levoglucosan. The standard solutions for the calibration curves

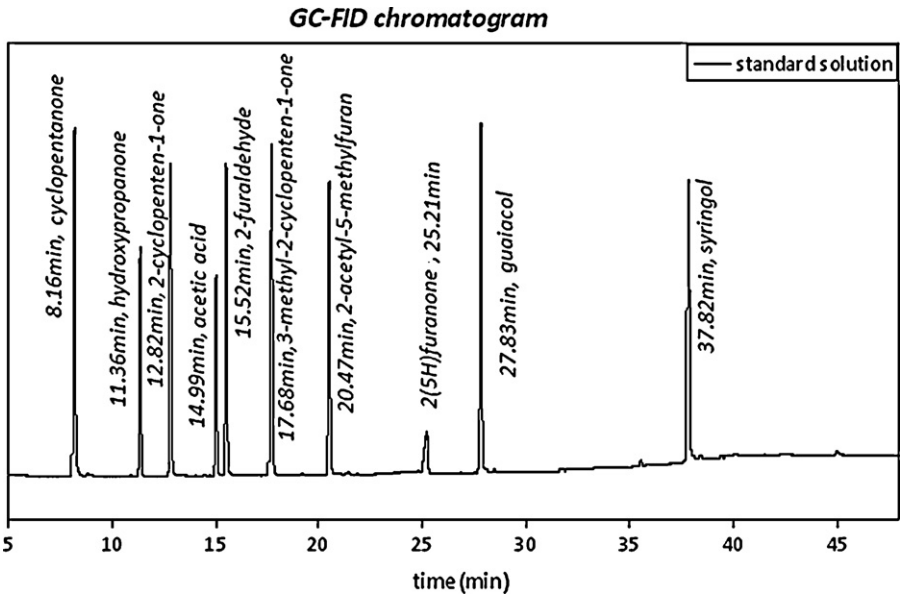

Fig. 1. GC-FID chromatogram of an aqueous standard solution.

were prepared by subsequent dilutions of each mother solution in acetone and dichloromethane respectively.

2.2. Sample preparation

Three different crude bio-oil samples were used. The water content of the samples ranged from 20 to 30 wt.%, carbon content ranged from 40.8 to 44.0 wt.%, hydrogen content from 7.7 to 8.4 wt.% and oxygen content from 48.2 to 50.8 wt.%.

For the GC × GC–TOFMS analysis samples were not pre-treated, but only diluted in acetone and filtered through a membrane filter with pore diameter 0.45 μm. The samples were filtered in order to eliminate any insoluble residual. Afterwards, each bio-oil was extracted with twice its volume with water and the aqueous phase was collected. This was subsequently analyzed by GC-FID, after proper dilution. In order to compare further the obtained quantitative and qualitative results with the GC × GC–TOFMS, the aqueous phase was extracted with dichloromethane and then analyzed.

2.3. GC-FID analysis

Chromatographic analyses were carried out with a HP5890II gas chromatograph, equipped with an FID. The column used was a DB-WAX 30 m × 0.53 mm × 1 μm. The carrier gas was Helium at a flow rate of 2 mL/min. Injections (0.4 μL) were made in the splitless mode at an injector temperature of 200 °C. The detector temperature was set at 235 °C. The temperature program employed was: initial oven temperature at 80 °C increasing at 4 °C/min up to 140 °C, stable at 140 °C for 2 min, then at 4 °C/min up to 225 °C and kept constant until the end of program. The total run time was 48.25 min (Fig. 1).

2.4. GC × GC–TOFMS analysis

The GC × GC analytical system was an Agilent 7890 A GC with injector Agilent 7683 B series (Agilent Technologies, Palo Alto, CA, USA) connected to a Pegasus 4D time-of-flight mass spectrometer from Leco Instruments (St. Joseph, MI, USA). The first dimensional chromatographic separation was performed by an apolar column VF-5MS (5% phenyl in polydimethyl-silicone; PDMS); 30 m; I.D. 0.25 mm, d.f. 0.25 μm. The second dimensional column was situated in a secondary internal oven and consisted of a phenyl (50%) PDMS column VF-17MS; 1.65 m; I.D. 0.10 mm, d.f. 0.20 μm. (extra 0.20 m of this column were used as a transfer line) both from Varian (Middelburg, The Netherlands). Cryofocusing by liquid nitrogen and a quad jet dual stage modulator (Zoex, Houston, TX, USA) was applied. Instrument control, data acquisition and data processing were done by the ChromaToF (Leco) software and Microsoft Excel.

The TOFMS operated at an acquisition rate of 100 spectra/s and a mass range of m/z 45–400 amu. The modulation period was 10 s. The carrier gas (He grade 5) flow rate was 1 mL/min; split injection of 0.2 μL sample solution at a split ratio of 1:40 and an injection temperature of 250 °C. Temperature programming was performed at an initial temperature of 35 °C of the primary GC oven and was kept stable for 10 min. Then the temperature increased at a rate of 3 °C/min up to 250 °C, followed by an increase at the rate of 15 °C/min to the final temperature of 300 °C, where it was kept stable until the end of the program. Total run time was 87.0 min. The secondary oven was programmed 15 °C ahead of the primary GC oven gradient. Modulator temperature offset was 30 °C.

2.5. External calibration method

For the purpose of quantification the external calibration method was followed. The model of calibration followed was that of weighted least squares linear regression. The slope and the intercept calculated are represented by the equations $a \pm t_{(n-2)}s_a$ and $b \pm t_{(n-2)}s_b$ were the t-value is taken at 95% confidence level and $(n-2)$ degrees of freedom, while s_a represents the standard deviation of the slope and s_b represents the standard deviation of the intercept.

3. Results and discussion

3.1. GC × GC–TOFMS analysis

3.1.1. Solvent choice and chromatographic conditions

A primary goal of this work was to establish a routine basis set of analysis in order to evaluate the usefulness and applicability of each procedure.

For the analysis of the untreated bio-oils, a proper solvent should be chosen that would not interfere with the early eluting com-

pounds at the GC × GC–TOFMS analysis and would solubilize the bio-oil quantitatively. To this end, tetrahydrofuran, acetone, chloroform, dichloromethane and ethyl acetate were tested. The bio-oil is almost completely soluble in tetrahydrofuran, ethyl acetate and chloroform, but these solvents elute together with low molecular weight acids, interfering with the analysis. Acetone offers good solubility and could be easily distinguished from acetic acid, an abundant bio-oil component. Finally, dichloromethane is a less polar solvent, which is a drawback for the solubilization of the polar bio-oils, nevertheless is sufficiently separated from the early eluting peaks.

Based on these observations, acetone was used as solvent for crude bio-oil samples and dichloromethane as an extraction solvent for the bio-oil analytes from the aqueous phase.

The injection temperature was tested in a range from 250 °C to 290 °C with no significant change and for this reason the 250 °C was maintained as a common setting for all the analysis. In order to improve the separation a VF-5MS column with 5% phenyl in 95% PDMS phase was used for the 1st dimension and a VF-17MS column with phenyl/PDMS 50:50 phase for the 2nd dimension. This combination is not truly orthogonal but still the significant difference of the polarity between the two columns enhances the separation of the analytes as in the 1st dimension the separation is based mostly on the volatility of the analytes while at the 2nd dimension the separation mechanism is based on interaction of analytes with the stationary phase (activity coefficient).

3.1.2. Data acquisition – processing, group type classification approach

Data acquisition took place under the ChromaToF software features. The raw data were processed by data processing methods developed by using the software properties, resulting in total ion chromatogram (TIC) and extracted ion chromatogram (EIC) for each sample. For the group type classification process these data were extracted in "csv" file format and processed further by Microsoft Excel for statistical evaluation. The deconvoluted spectra were compared with the NIST library software for correct matching. After comparison, only peaks with similarity more than 700 were named.

At first a data processing method for identification of all peaks was ran for presentation in a 2D contour plot. Next, peak areas corresponding to solvent elution and column bleeding were "erased" using the features of ChromaToF. Thereafter a new data processing (DP) method acquiring the most abundant-unique ion illustrated finally a unique ion chromatogram (UIC) contour plot representing peak areas cohering the 100% of relative peak area distribution. These peak area values of extracted trace ions were used later for quantification purposes. With the procedure described above peak tables containing an average of 300 compounds were acquired as a result of a number of factors such as dilution ratio of sample with solvent, injection volume, split ratio, solvent selectivity, signal to noise criterion for peak finder in DP method, as well as detector and column conditions. In Fig. 2 a 3D plot of the visualization features of ChromaToF is depicted.

Group type classification eventuated in two steps. In the first step, seven major groups were designated by borderline group-cells on the 2D contour plot of the first sample. The groups were defined as follows: 1. Acids & esters, 2. Aldehydes & ketones, 3. Aromatic hydrocarbons, 4. Hydrocarbons, 5. Phenolics, 6. Sugars and 7. "Not classified". Noteworthy is the fact that furans were eluted in the area of the 2nd group, "Aldehydes & ketones". For this reason, a separate area for furans was defined in a second step. Correspondingly, at the same chromatographic region of the "Phenolics" group, guaiacols and syringols or in general, methoxy phenolic derivatives were eluted, that could constitute a separate classification group. A good explanation for the aforementioned behavior is the chemical structure similarity of these compounds despite the fact that

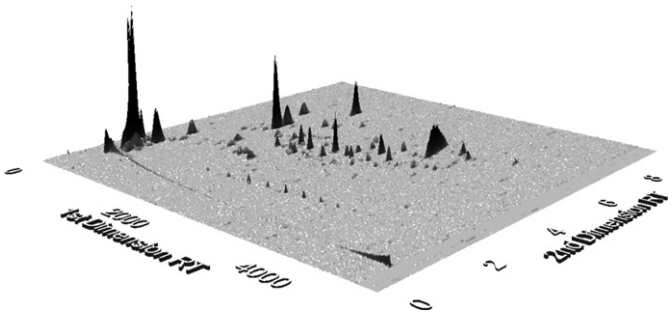

Fig. 2. 3D surface plot of bio-oil #1.

these compounds belong to different chemical classes. In addition, polarity and boiling points are quite similar in these cases. A similar result occurs in the characteristic elution area of acetic acid. At the same broad-range elution area of acetic acid, it is possible for 6 more compounds to be detected like formic acid, 2-butanone, 2-butenal, 2,3-butanedione, methyl vinyl ketone and 2-methyl furan. The identification of these compounds, prove the great deconvolution power of the software. The greatest the concentration of acetic acid in bio-oil samples, the broader the elution range and thus more convoluted peaks arise. On the other hand, sugars region is distinct enough. By comparing the 1st with the other two samples and simple adjustments, the borderlines seemed to be appropriate for all samples. The borders were determined empirically by checking almost all compounds occurring near the borderlines of each group and by EIC [19].

In the second step, two more groups were added as a consequence of determination of "furans group" by correcting the results of group type classification in the first step. This was accomplished by checking every peak one by one in the first classification data. The second group was generated by splitting the "Not classified" group in two groups: the "Unidentified" and the "Not classified" groups. The "Unidentified" group consisted of compounds with low similarity or false matching hits with NIST library software. After this correction the "Not classified" group consisted of all those compounds that were identified by the software but constituted other smaller individual groups like amines, dioxolanes, alcohols, etc., which were not further classified. For the former corrections apart from similarity, expected retention time was considered for several compounds.

3.1.3. Identification

Initially, the total peak areas of the solvent and the column bleeding were removed and the rest peak areas of all analytes were converted to the new relative area contribution representing the 100% of relative peak areas.

At a first glance, data were processed using the TIC to get a brief description of the samples composition and to keep that data for conclusive comparison at the end of the present work. In order to isolate the most abundant analytes using information obtained by TIC, a selection was performed as proposed by Marsman et al. [19], by setting a minimum relative peak area criterion. By using these considerations, four classes of summed relative peak areas arose (Table 1).

The first class with relative peak area greater than 1% resulted in the top 10–20 compounds being present in these three different samples (Table 1). The second and third class consisted of analytes with relative peak area greater than 0.5% and 0.3% respectively, while the last one consisted of peaks with relative area % greater than 0.3 plus analytes with similarity greater than 850 and relative area % greater than 0.1.

Table 1
Number of peaks according to minimum peak areas.

Criteria	Number of peaks detected		
	Bio-oil #1	Bio-oil #2	Bio-oil #3
Total number of detected peaks (between brackets: relative area)	234 (100%)	230 (100%)	243 (100%)
Peak area > 1%	18 (64.4%)	18 (65.6%)	22 (64.2%)
Peak area > 0.5%	30 (73.0%)	36 (78.3%)	37 (74.8%)
Peak area > 0.3%	57 (83.4%)	51 (83.7%)	58 (82.9%)
Peak area > 0.3% plus similarity > 850 and peak area > 0.1%	98 (90.2%)	93 (91.1%)	98 (90.3%)

Table 2
Groups for classification before and after correction.

Group no.	Before correction[a]	Group no.	After correction[b]
1	Acids & esters	1	Acids & esters
2	Aldehydes & ketones	2	Aldehydes & ketones
		3	Furans
3	Hydrocarbons	4	Hydrocarbons
4	Aromatic hydrocarbons	5	Aromatic hydrocarbons
5	Phenolics	6	Phenolics
6	Sugars	7	Sugars
7	Not classified	8	Not classified
		9	Unidentified

[a] As a result of group type borderline classification.
[b] As a result of correcting the obtained peak tables in excel files. The incorrectly nominated peaks were designated to the appropriate classification groups.

3.1.4. Classification and data processing procedure

In a first stage, seven major different groups were defined for the classification of these three bio-oils, as shown below (Table 2).

The border line groups are depicted in Figs. 3–5 for the three bio-oil samples respectively.

In several cases, or more specifically groups, many analytes belonging to different chemical functional groups elute in neighboring retention times. This leads to many false analyte to group designations. By defining wide-content areas like the second group the problem of correction between furans, cyclic or linear ketones and aldehydes was not solved but overlooked. On the other hand, the definition of more groups like amines or alcohols is necessary for more detailed characterization of the oils composition. After consideration of the complexity of the samples and following the strategy to create as few as possible "general" groups, no more groups were added. A future approach could implement all possible subgroups of chemical functionality in the larger "group areas" developing a highly complicated classification chromatographic map.

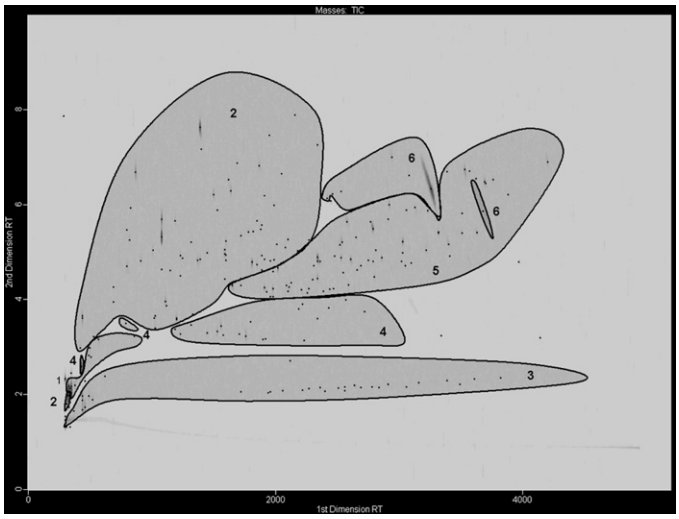

Fig. 3. Contour plot for bio-oil sample #1 diluted in acetone. Six groups are depicted with borderlines and the rest peaks constitute the group "Not classified".

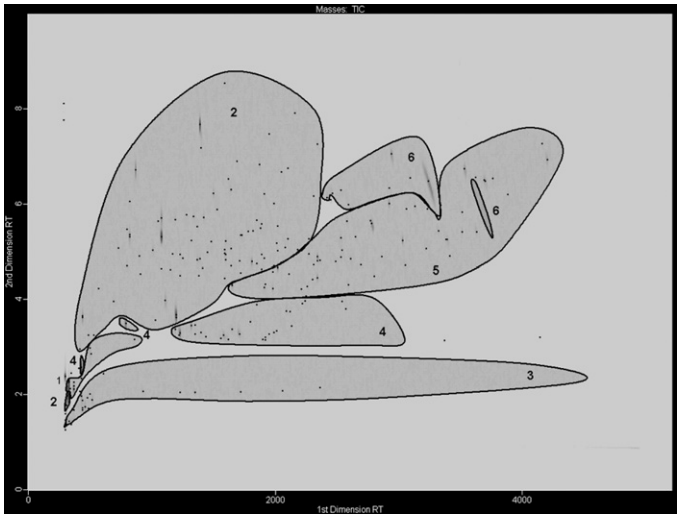

Fig. 4. Contour plot for bio-oil sample #2 diluted in acetone.

The data obtained by the analysis of the three bio-oil samples diluted in acetone, were processed further for correction of classification results. For these three samples, all the identified compounds in the borderlines were checked one by one in each group and were corrected by assigning each compound to the proper group (Table 3).

This procedure is quite laborious and thus this level of correction was applied only in the aforementioned samples in order to truly check the level of correctness for the "first step" classification procedure. To simplify the procedure the selection according

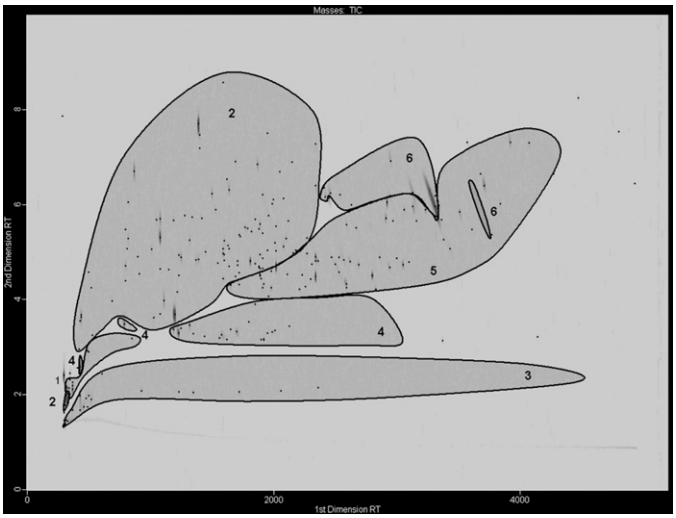

Fig. 5. Contour plot for bio-oil sample #3 diluted in acetone.

Table 3
Group type composition based on relative peak area percentage.

Group no.	Classification group	Sample		
	Rel. area % of group[a], number of peaks[b], correct hits[c]	Bio-oil #1	Bio-oil #2	Bio-oil #3
1	Acids & esters	23%	19%	11%
	n	22	28	27
	Correctness	73%	42%	60%
2	Aldehydes & ketones	19%	14%	13%
	n	35	34	41
	Correctness	67%	56%	55%
3	Furans	10%	9%	11%
	n	20	23	24
	Correctness	–	–	–
4	Hydrocarbons	6%	4%	4%
	n	30	19	21
	Correctness	94%	80%	90%
5	Aromatic hydrocarbons	5%	9%	8%
	n	20	21	20
	Correctness	81%	64%	87%
6	Phenolics	13%	11%	17%
	n	47	38	36
	Correctness	65%	62%	53%
7	Sugars	13%	7%	17%
	n	6	9	8
	Correctness	71%	88%	72%
8	Not classified	7%	21%	13%
	n	31	31	33
	Correctness	–	–	–
9	Unidentified	3%	6%	5%
	n	23	27	33
	Correctness	–	–	–

[a] First line, the name and relative area percentage (%) of each group is shown.
[b] Second line, the number of components (*n*) in each specified group between brackets.
[c] Third line indicates the percentage of number of correct hits related to the total number of peaks for each group. Groups no. 3, 8 and 9 were defined after correction and for this reason there is no meaning in a value of correct hits.

to Marsman et al. [19] could be followed, describing the 90% of total relative peak area with only 90 peaks as presented in the fourth category in Table 1. In this case, checking about 90 peaks is more convenient without significant data loss.

After correction, two more groups were defined increasing the number of total groups to nine (Table 2). The level of correct hits was between 42% and 94% depending on the group. For example hydrocarbons and sugars were all in all three cases at good level of correction since they possess specific areas where rarely other compounds co-elute. It was also difficult to separate two discrete areas for guaiacols and syringols. Thus, these two groups were integrated in the wider "Phenolics" group. The remaining compounds were defined as "Not classified" (Table 3).

In the corrected classified results, all furans, which were found in the area of "Aldehydes & Ketones", were defined as a new group: "Furans". The group "Not classified" consisted mostly of nitrogen containing compounds like amines and oxygen containing compounds like dioxolanes at a level of 2–5% of relative area. At about the same percentage existed compounds, which were not identified by the ChromaToF software due to the low S/N ratio.

A histogram of relative area percentages for every classification group is depicted in Fig. 6.

3.2. Quantification of the bio-oil by GC-FID and GC × GC–TOFMS

3.2.1. Linearity range

The linearity range of the calibration curve for each compound is presented in Table 4. The correlation coefficient provides an estimate of the linearity of the data, but suffers from uncertainty. For this reason the *F*-test of residual standard deviation against repeatability standard deviation is employed, according to the equation

$$F = \frac{s_{y/x}^2}{s_r^2}$$

This is a one-tailed test at the 95% confidence level, and from the corresponding tables the critical values of *F* for the measurements presented are 5.192 and 6.591 for the GC-FID and the GC × GC–TOFMS respectively. The null hypothesis for this test is that $s_{y/x} = s_r$. According to this test if the calculated value of *F* is smaller than the critical value, then the null hypothesis is rejected and the linearity is supported [31]. The standard deviation of the measurements of the standard solution, with concentrations near the centroid of all the points, was chosen for the calculation of the *F* value for each compound, and the results are also presented in Table 4. Additionally, the residuals were plotted against concentration, and were found to be randomly scattered verifying the linearity of the calibration curves in the examined range.

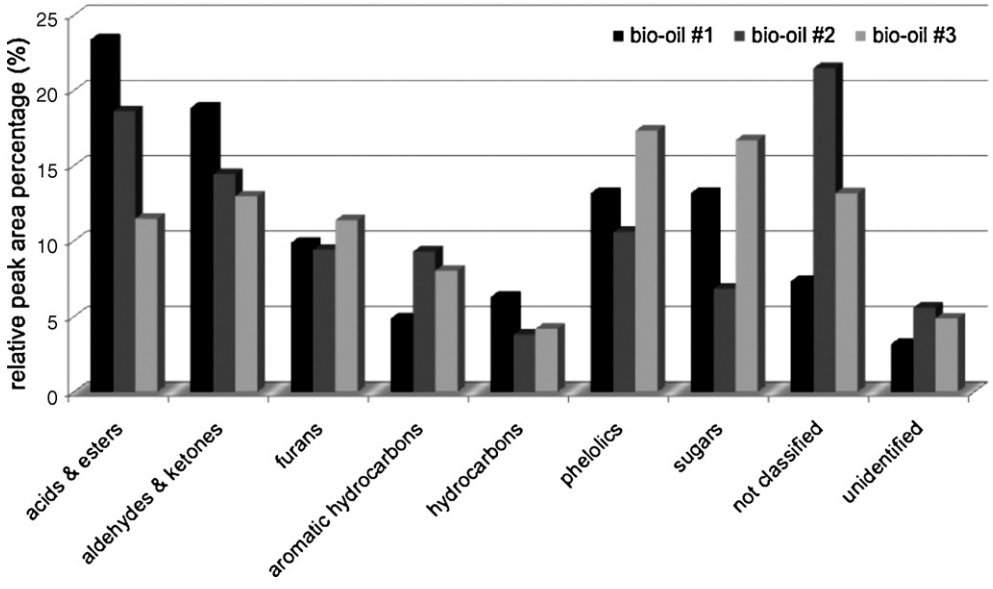

Fig. 6. Histogram for comparison of the 3 bio-oil samples composition based on total relative peak area of the classification groups (1–9).

Table 4
Linearity range of the calibration curves for the GC-FID and GC × GC–TOFMS analysis.

Compound	GC-FID				GC × GC–TOFMS			
	Linearity range (μg g^{-1})		r	F	Linearity range (μg g^{-1})		r	F
	Min	Max			Min	Max		
Cyclopentanone	16.5	319.4	0.9994	0.007	8.9	107.4	0.9985	0.371
Hydroxypropanone	23.8	461.2	0.9997	0.175	32.3	427.2	0.9983	0.403
2-Cyclopenten-1-one	18.0	350.2	0.9995	0.186	9.7	155.8	0.9988	0.111
Acetic acid	33.2	643.9	0.9995	0.098	29.2	443.7	0.9964	0.165
2-Furaldehyde	24.5	474.9	0.9997	0.178	13.8	164.9	0.9969	0.041
3-Methyl-2-cyclopenten-1-one	17.8	345.1	0.9997	0.213	12.0	112.0	0.9984	0.113
2-Acetyl-5-methylfuran	19.8	384.3	0.9998	0.270	10.0	120.5	0.9998	0.019
2(5H)Furanone	20.6	399.7	0.9997	0.044	14.4	217.4	0.9997	0.223
Guaiacol	19.5	379.2	0.9998	0.024	15.5	233.4	0.9987	0.472
Syringol	21.3	413.4	0.9999	0.003	7.8	260.7	0.9999	0.050
Levoglucosan	–	–	–	–	17.6	267.4	0.9993	0.818

3.2.2. Weighted linear regression analysis

The main models employed for the manipulation of experimental data for the generation of the calibration curves are the weighted least squares linear regression (WLSLR) and the linear least squares regression model (LSLR). The LSLR has been more widely used due to the simplicity of its calculations however it assumes homoscedasticity of the data, which does not always reflect the reality. In fact, the data are usually heteroscedastic thereby contributing with different standard deviations to the calibration curve. Most commonly in chromatographic experiments, the error of the measured data increases with the concentration of the analyte. In the WLSLR model the regression line includes the different weight of each point and in the end provides more realistic results about the errors and confidence limits of the calculated concentrations [31–33]. The weighting factor is usually the inverse of the corresponding variance (σ_i^{-2}) for each calibration point, but other more empirical weights have also been proposed such as $1/x$, $1/x^{1/2}$, $1/x^2$, $1/y$, $1/y^{1/2}$, $1/y^2$. In this work the inverse of variance has been used as a weighting factor.

The calibration curve data were fitted to the WLSLR model, in order to obtain the calibration equations for each compound. The equation followed was the $y = a + bx$. The standard deviations of the slope (b) and the intercept (a) were calculated at a 95% confidence level. The correlation coefficients were above 0.998 and 0.996 for the GC-FID and GC × GC–TOFMS respectively, for all the compounds. Additionally, the values of s_b/b were calculated in order to confirm that their value was less than 5%, as is required by the validation condition [34,35]. The results are presented in Table 5

The limits of detection and quantification for each standard compound were calculated from the parameters of the generated calibration curves, based on the following equations and are presented in Table 5

$$\mathrm{LD} = 3.3 \times \frac{s_a}{b} \quad \text{and} \quad \mathrm{LQ} = 10 \times \frac{s_a}{b}$$

Both methods offer satisfactorily low limits of detection and quantification, and as was expected, the corresponding limits for the GC × GC–TOFMS method are lower due to its inherent greater sensitivity.

Additionally, the precision and accuracy of the GC-FID method were estimated, in order to support further the use of this method for the rapid screening of bio-oil samples, prior to their full analysis with the more laborious and costly GC × GC–TOFMS method. The inter- and intra-day precision, along with the accuracy were calculated at three concentration levels ($n = 3$), while the instrumental precision was also calculated at the medium concentration level. The inter- and intra-day precision ranged from 1.61 to 12.49% and from 1.68 to 9.69% respectively, while the accuracy ranged from 9.31 to 11.18%. All the values calculated are below ±15%, which

Table 5
Calibration parameters of the GC-FID and the GC × GC–TOFMS analysis.

Compound	a	$t_{(n-2)} \times s_a$	b	$t_{(n-2)} \times s_b$	s_b/b (%)	LD (μg g^{-1})	LQ (μg g^{-1})
GC-FID							
Cyclopentanone	4284.3	4031.5	1574.2	73.5	1.7	3.0	9.2
Hydroxypropanone	3527.9	2235.3	620.7	18.3	1.1	4.3	13.0
2-Cyclopenten-1-one	44446.6	4195.1	1442.6	60.6	1.5	3.5	10.5
Acetic acid	11747.8	4783.8	400.0	17.5	1.6	14.2	43.0
2-Furaldehyde	4114.3	3854.3	988.4	32.8	1.2	4.6	14.0
3-Methyl-2-cyclopenten-1-one	5183.8	4462.3	1530.3	47.3	1.1	3.5	10.5
2-Acetyl-5-methylfuran	4619.6	4647.5	1196.5	33.0	1.0	4.6	14.0
2(5H)Furanone	161.0	4238.0	858.6	25.4	1.1	5.9	17.8
Guaiacol	5443.3	4340.6	1401.8	32.6	0.8	3.7	11.1
Syringol	3836.3	2675.8	1027.5	8.8	0.3	3.1	9.4
GC × GC–TOFMS							
Cyclopentanone	−18613.1	60363.4	23529.6	2339.7	3.1	2.7	8.1
Hydroxypropanone	−9441.8	7813.5	806.7	86.4	3.4	10.1	30.5
2-Cyclopenten-1-one	−21201.3	29092.2	10972.0	981.0	2.8	2.8	8.3
Acetic acid	−61405.1	39016.0	5237.2	431.3	2.6	7.7	23.4
2-Furaldehyde	−79738.6	211274.3	17548.2	1800.7	3.2	12.6	38.2
3-Methyl-2-cyclopenten-1-one	−11404.0	23165.6	4971.9	642.8	4.1	4.8	14.7
2-acetyl-5-Methylfuran	−37938.8	20001.5	13682.1	375.6	0.9	1.5	4.6
2(5H)Furanone	−68084.5	49684.5	13519.3	506.9	1.2	3.8	11.6
Guaiacol	−9933.2	14175.5	4598.6	374.7	2.6	3.2	9.7
Syringol	−3413.3	1293.3	1888.2	17.8	0.3	0.7	2.2
Levoglucosan	−8030.4	7800.3	3761.0	316.4	2.7	2.2	6.5

Table 6
Quantitative analysis of the samples (diluted in acetone) by the GC × GC–TOFMS method.

Compound	GC × GC–TOFMS analysis		
	Bio-oil 1 (wt.%)	Bio-oil 2 (wt.%)	Bio-oil 3 (wt.%)
Cyclopentanone	0.03 ± 0.002	0.02 ± 0.004	0.02 ± 0.003
Hydroxypropanone	2.1 ± 0.03	2.2 ± 0.2	3.7 ± 0.2
2-Cyclopenten-1-one	0.2 ± 0.02	0.1 ± 0.02	0.1 ± 0.01
Acetic acid	5.9 ± 0.5	7.4 ± 0.3	4.6 ± 0.5
2-Furaldehyde	0.4 ± 0.04	0.4 ± 0.04	0.3 ± 0.03
3-Methyl-2-cyclopenten-1-one	0.1 ± 0.01	0.04 ± 0.01	0.05 ± 0.01
2-Acetyl-5-methylfuran	0.01 ± 0.004	0.01 ± 0.001	0.01 ± 0.002
2(5H)Furanone	0.4 ± 0.02	0.7 ± 0.09	0.7 ± 0.05
Guaiacol	0.2 ± 0.01	0.2 ± 0.02	0.6 ± 0.06
Syringol	0.3 ± 0.03	0.3 ± 0.04	0.02 ± 0.01
Levoglucosan	4.1 ± 0.3	2.7 ± 0.2	6.2 ± 0.2

Table 7
Quantitative results of the samples (aqueous phase) by GC-FID and the GC × GC–TOFMS analysis. The results refer to the bio-oil sample.

	Bio-oil 1 (wt.%)		Bio-oil 2 (wt.%)		Bio-oil 3 (wt.%)	
Compound	GC × GC–TOFMS	GC-FID	GC × GC–TOFMS	GC-FID	GC × GC–TOFMS	GC-FID
Cyclopentanone	0.01 ± 0.0004	ND	0.01 ± 0.001	ND	0.01 ± 0.002	ND
Hydroxypropanone	0.47 ± 0.01	1.33 ± 0.07	0.55 ± 0.004	1.77 ± 0.06	0.62 ± 0.13	2.20 ± 0.04
2-Cyclopenten-1-one	0.08 ± 0.0003	0.10 ± 0.05	0.06 ± 0.003	0.09 ± 0.01	0.04 ± 0.007	0.10 ± 0.01
Acetic acid	0.54 ± 0.04	3.63 ± 0.29	0.82 ± 0.04	6.63 ± 0.51	0.32 ± 0.09	3.78 ± 0.41
2-Furaldehyde	0.15 ± 0.01	0.12 ± 0.02	0.17 ± 0.02	0.19 ± 0.02	0.08 ± 0.02	0.09 ± 0.01
3-Methyl-2-Cyclopenten-1-one	0.02 ± 0.001	ND	0.02 ± 0.001	ND	0.01 ± 0.003	ND
2-Acetyl-5-methylfuran	0.003 ± 0.0001	ND	0.004 ± 0.0001	ND	0.003 ± 0.0001	ND
2(5H)Furanone	0.21 ± 0.01	0.05 ± 0.004	0.35 ± 0.04	0.24 ± 0.02	0.40 ± 0.10	0.29 ± 0.03
Guaiacol	0.04 ± 0.002	0.04 ± 0.005	0.05 ± 0.003	0.04 ± 0.004	0.15 ± 0.03	0.17 ± 0.01
Syringol	0.10 ± 0.003	0.10 ± 0.02	0.15 ± 0.01	0.14 ± 0.01	0.001 ± 0.001	0.008 ± 0.001

is the acceptable RSD for these measurements [36,37]. Lastly, the instrumental precision had an average of 7.91%.

3.3. Samples analysis

Three bio-oil samples were analyzed qualitatively and quantitatively by means of GC × GC–TOFMS, without any pretreatment apart from an appropriate dilution in acetone and subsequent filtration. Afterwards, the original samples were extracted with water, and the aqueous phase was separated and injected to the GC-FID system, without any pretreatment apart from proper dilution. The same aqueous phase was extracted with dichloromethane and the organic phase was injected to the GC × GC–TOFMS system. The quantitative results of all three measurements are presented in Tables 6 and 7.

Regarding Tables 6 and 7, it is deduced that there is a good correlation between the results of the GC × GC–TOFMS analysis of the crude bio-oil and the GC-FID analysis of the aqueous phase. It should be mentioned that the results of the aqueous phase analysis were calculated so as to refer to the crude bio-oil, in order to have a direct comparison between the results. Extraction of the analytes is in any case not quantitative and using different water to bio-oil ratio, or a different extraction temperature could improve their partitioning towards water and could subsequently improve the agreement of the results. Nonetheless, most of the analytes examined are extracted in the aqueous phase at detectable amounts, in accordance to their polarity. The quantitative analysis of the same aqueous phase with the GC × GC–TOFMS system resulted in lower concentrations. The reason for this is probably that the analytes selected for the quantification are mostly polar and therefore are not adequately extracted with the non-polar dichloromethane. This is especially evident in the case of levoglucosan, which was found in the bio-oil in ample concentration, but since it is completely insoluble in dichloromethane, it was not detected in the dichloromethane extract of the aqueous phase. In conclusion, the quantitative analysis of the aqueous phase with the GC × GC–TOFMS can only provide for qualitative results about the analytes present. The presented GC-FID method, with a polar column, is a lot more cost efficient and even though it has the limitations of 1D separations it provides a reliable estimation of the concentration of the selected analytes, thereby being the proposed method when the investigation of the quantity of a few target compounds in aqueous samples is needed.

4. Conclusion

It is concluded that in special cases with complex mixtures special attention should be paid to all parameters of analysis. Especially when solvent extraction techniques are interfering with the analysis set. In the identification section, it is essential the fact that the majority of a sample is described, representing the 80–95% of the total relative percentage area. This was not accomplished so far with one-dimensional GC systems and further development in comprehensive techniques will further enhance this percentage. Identification is a less laborious work in comparison to classification and quantification. It can be easily managed, to keep reliable results by selecting peaks with relative area > 0.3% in addition to peaks with similarity > 850 plus relative area > 0.1%. By fulfilling the above conditions (the number of peaks is reduced), a total relative area of 90–92% is still represented, thus providing a representative picture of the sample.

Classification for bio-oil samples is a problematic procedure in some cases due to the high complexity of the samples and the structure–activity similarity of specific compounds. By borderline group type classification 7 groups could be separated, which after correction of the data were transformed to 9 groups by distinguishing “Furans” and “Unidentified” in separate groups. This classification map with 7 groups was created for one sample and then was applied to the rest. It showed good matching as the parameters of the classification were kept the same and the corrections needed were at a minimum level. That supports the suggestion that a classification method developed on a GC × GC–TOFMS system could be adjusted to a GC × GC-FID system providing remarkable

group quantification results. There is a lot of future work in the development of better classification methods and especially in such complex mixtures like pyrolysis oils. A more elaborate classification method that would be based on different criteria such as retention times in both dimensions, border-line groups, similarity with libraries and compound names, would be of significant interest.

The WLSLR model was successfully used for describing the calibration curves for both the chromatographic methods proposed. The linearity range of both methods for the selected compounds was satisfactory and within the concentration limits expected to be found in a given bio-oil. The LDs and LQs of both methods were comparable and sufficiently low. Three different bio-oil samples were analyzed by the GC × GC–TOFMS and by using the generated calibration curves, their concentration in the selected analytes was determined. The same bio-oils were extracted with water and the analysis of this aqueous phase by the GC-FID method rendered comparable results. The accuracy and precision of the GC-FID method have also been demonstrated, thereby supporting that this method is reliable and can be used for the analysis of the bio-oils. Both methods assist to a better comprehension of the effect of pyrolysis conditions and biomass type, on the bio-oils' composition.

References

[1] D.K. Shen, S. Gu, Bioresour. Technol. 100 (2009) 6496.
[2] D.D. Das, M.I. Schnitzer, C.M. Monreal, P. Mayer, Bioresour. Technol. 100 (2009) 6524.
[3] M. Garcia-Perez, T.T. Adams, J.W. Goodrum, D.P. Geller, C.C. Das, Energy Fuels 21 (2007) 2363.
[4] M. Ikura, M. Stanciulescu, E. Hogan, Biomass Bioenergy 24 (2003) 221.
[5] D. Mourant, D.Q. Yang, X. Lu, C. Roy, Wood Fiber Sci. 73 (2005) 542.
[6] Z. Qi, C. Jie, W. Tiejun, X. Ying, Energy Convers. Manage. 48 (2007) 87.
[7] S. Czernik, A.V. Bridgwater, Energy Fuels 18 (2004) 590.
[8] D. Mohan, C.U. Pittman, P.H. Steele, Energy Fuels 20 (2006) 848.
[9] A.A. Lappas, M.C. Samolada, D.K. Iatridis, S.S. Voutetakis, I.A. Vasalos, Fuel 81 (2002) 2087.
[10] S. Stephanidis, C. Nitsos, K. Kalogiannis, E.F. Iliopoulou, A.A. Lappas, K.S. Triantafyllidis, Catal. Today, submitted for publication.
[11] D. Mohan, J. Shi, D.D. Nicholas, C.U. Pittman Jr., P.H. Steele, J.E. Cooper, Chemosphere 71 (2008) 456.
[12] C.A. Mullen, A.A. Boateng, N.M. Goldberg, I.M. Lima, D.A. Laird, K.B. Hicks, Biomass Bioenergy 34 (2010) 67.
[13] A. Oasmaa, D. Meier, J. Anal. Appl. Pyrol. 73 (2005) 323.
[14] N. Özbay, B.B. Uzun, E.A. Varol, A.E. Pütün, Fuel Process. Technol. 87 (2006) 1013.
[15] M. Garcia-Perez, A. Chaala, H. Pakdel, D. Kretschmer, C. Roy, J. Anal. Appl. Pyrol. 78 (2007) 104.
[16] C. Branca, P. Giudicianni, C. Di Blasi, Ind. Eng. Chem. Res. 42 (2003) 3190.
[17] A. Fullana, J.A. Contreras, R.C. Striebich, S.S. Sidhu, J. Anal. Appl. Pyrol. 74 (2005) 315.
[18] J.H. Marsman, J. Wildschut, F.H. Mahfud, H.J. Heeres, J. Chromatogr. A 1150 (2007) 21.
[19] J.H. Marsman, J. Wildschut, P. Evers, S. de Koning, H.J. Heeres, J. Chromatogr. A 1188 (2008) 17.
[20] M. Windt, D. Meier, J.H. Marsman, H.J. Heeres, S. de Koning, J. Anal. Appl. Pyrol. 85 (2009) 38.
[21] W.T. Tsai, J.H. Chang, K.J. Hsien, Y.M. Chang, Bioresour. Technol. 100 (2009) 406.
[22] W.T. Tsai, M.K. Lee, J.H. Chang, T.Y. Su, Y.M. Chang, Bioresour. Technol. 100 (2009) 2650.
[23] R. Bayerbach, D. Meier, J. Anal. Appl. Pyrol. 85 (2009) 98.
[24] J. Dalluge, J. Beens, U.A.Th. Brinkman, J. Chromatogr. A 1000 (2003) 69.
[25] M. Adahchour, J. Beens, U.A.Th. Brinkman, J. Chromatogr. A 1186 (2008) 67.
[26] P.L. Desbene, M. Essayegh, B. Desmazieres, F. Villeneuve, J. Chromatogr. A 553 (1991) 211.
[27] O. Amador-Munoz, P.J. Marriott, J. Chromatogr. A 1184 (2008) 323.
[28] L.A.F. de Godoy, E.C. Ferreira, M.P. Pedroso, C.H. de, V. Fidelis, F. Augusto, R.J. Poppi, Anal. Lett. 41 (2008) 1603.
[29] K.M. Pierce, J.C. Hoggard, R.E. Mohler, R.E. Synovec, J. Chromatogr. A 1184 (2008) 341.
[30] F.H. Mahfud, F.P. van Geel, R.H. Venderbosch, H.J. Heeres, Sep. Sci. Technol. 43 (2008) 3056.
[31] A.M. Almeida, M.M. Castel-Branco, A.C. Falcao, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 774 (2002) 215.
[32] M.J. Cardone, S.A. Willavize, M.E. Lacy, Pharm. Res. 7 (1990) 154.
[33] J. Neter, W. Wasserman, M.H. Kutner (Eds.), Applied Linear Regression Models, Richard D. Irwin, Inc., Illinois, 1983.
[34] C. Mansilha, A. Melo, H. Rebelo, I.M.P.L.V.O. Ferreira, O. Pinho, V. Domingues, C. Pinho, P. Gameiro, J. Chromatogr. A 1217 (2010) 6681.
[35] J.N. Miller, J.C. Miller (Eds.), Statistics and chemometrics for Analytical Chemistry, Pearson Education Limited, England, 2005.
[36] M. Yuwono, G. Indrayanto, Profiles Drug Subst. Excipients Relat. Methodol. 32 (2005) 243.
[37] EURACHEM/CITAC, Guide, Quantifying Uncertainty in Analytical Measurement, 2000.