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Absolute quantitation of lignin pyrolysis products using an internal standard

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Abstract

A simple method to obtain absolute quantitation of lignin pyrolysis fragments by on-line pyrolysis–gas chromatography–mass spectrometry (Py–GC–MS) is proposed. Three compounds were tested as internal standards, i.e. 1,3,5-tri-*tert.*-butylbenzene, 1,2,4-benzenetricarboxylic acid trimethyl ester and 1,3,5-trimethoxybenzene. The characteristic of the proposed internal standards is that they vaporize in the hot pyrolysis interface during the 3-min equilibration and focus on the top of the GC column, thus avoiding loss of internal standard due to thermal fragmentation. The linearity and reproducibility of response of the standards over a range of concentrations are reported. 1,3,5-Tri-*tert.*-butylbenzene was selected as the most appropriate among the tested standards to be used in Py–GC–MS of lignin. The correction factors for the main lignin pyrolysis fragments were calculated and a practical application of the proposed method to the analysis of a wheat straw sample is discussed.

Keywords: Pyrolysis; Internal standards; Lignin; Tributylbenzene

1. Introduction

Lignin is one of the structural components of plant cell walls and provides strength and rigidity in plant tissues. Lignin is a high-molecular-mass polymer made up of three phenylpropanoid units derived from *p*-coumaryl, coniferyl and sinapyl alcohols. The phenyl moieties of these compounds are referred to as *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) units. These monomeric constituents are linked together to form a complex, tridimensional structure which is difficult to characterize. Analysis is performed by chemical or thermal degradation of the lignin macromolecule into smaller compounds which are separated by means of chromatographic techniques [1,2].

Analytical pyrolysis is a useful technique for the analysis of macromolecular and polymeric samples. Pyrolysis thermally degrades polymers into small fragments which are separated by gas chromatography and identified with the aid of mass spectrometry. The obtained pyrogram constitutes a fingerprint of the starting macromolecule and gives information on the relative amount of its monomeric components [3]. The great advantages of Py–GC–MS over other degradation techniques are the small

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sample size required and the fact that no sample pretreatment is necessay except grinding. Analytical pyrolysis is commonly used for the analysis of lignocellulosic materials and is of particular interest because it eliminates the workup required to chemically degrade lignocellulose. Py–GC–MS analysis can provide information on lignin classification on the basis of H, G and S ratio [4,5], chemical changes in cell wall structural components during plant maturation [6,7] and fingerprinting of various origin lignocellulosic materials, e.g., in paper industry effluents [8,9], feeds [10–12] and in forest litter [13].

Absolute quantification in analytical pyrolysis is seldom used. A few experiments have reported absolute quantitative results using an off-line system which requires the trapping of the pyrolysis products and their solubilization with a known amount of solvent. An internal standard is added to the solution which can then be injected in the gas chromatograph [5]. This rather complex procedure eliminates the most attractive aspect of Py–GC–MS: the minimal sample workup. On the other hand a practical, non time consuming, on-line method for absolute quantitation in Py–GC–MS analysis is badly needed because the lack of absolute quantification prevents Py–GC–MS data from being compared on an absolute scale with other analytical techniques.

In this paper, the use of an internal standard which can be added directly to the pyrolysis sample holder and used in on-line Py–GC–MS of lignin is studied. 1,3,5-Tri-*tert.*-butylbenzene, 1,2,4-benzenetricarboxylic acid trimethyl ester and 1,3,5-trimethoxybenzene were tested to verify their usefulness as internal standard. Their linearity of response and the analysis reproducibility were measured. The correction factors for some of the lignin pyrolysis fragments were calculated and the method was applied to the determination of lignin absolute amount in a wheat straw sample.

2. Experimental

2.1. Py–GC–MS

All analyses were performed using a CDS Pyroprobe 1000 (Chemical Data System, Oxford, PA, USA) heated filament pyrolyzer interfaced to a GC–MS instrument consisting of a Varian 3400 gas chromatograph (Varian Analytical Instruments, Walnut Creek, CA, USA) coupled to a Finnigan MAT (Finnigan MAT, San Jose, CA, USA) Magnum ion trap mass spectrometer. Pyrolysis was performed at 600°C/5 s and the Py–GC interface was set at 200°C. The GC column was a Supelco SPB-5 (Supelco, Bellefonte, PA, USA) (30 m×0.32 mm I.D., 0.25 μm film thickness) operated from 50 to 290°C at 5°C/min, holding the initial temperature for 10 min. The injector was set at 250°C in the split mode (1/100 split ratio). Mass spectra were recorded under electron impact at 70 eV from 40 to 400 m/z (1 scan/s).

2.2. Linearity and repeatability

1,3,5-Tri-*tert.*-butylbenzene, 1,2,4-benzenetricarboxylic acid trimethyl ester and 1,3,5-trimethoxybenzene were purchased from Aldrich Chimica (Milan, Italy). Solutions in CH_2Cl_2 of each standard in the 10–100 μg/ml range were carefully added to the quartz capillary tube (5 μl) which was immediately inserted into the Py–GC interface. After an equilibration period of 3 min, the pyrolysis at the desired temperature was performed.

2.3. Correction factors

Phenolic standards (2-methylphenol, guaiacol, 4-ethylphenol, 4-methylguaiacol, 2,6-dimethoxyphenol, eugenol, vanillin, acetovanillone, 4-allyl-2,6-dimethoxyphenol and syringaldehyde) were purchased from Aldrich. A 1-μl aliquot of a 0.2 mg/ml CH_2Cl_2 solution of phenolic standards and 1,3,5-tri-*tert.*-butylbenzene was injected in the GC–MS system (triplicate analysis) to calculate correction factors, all other experimental conditions being the same as described in Section 2.1.

3. Results and discussion

Preliminary studies on the reproducibility of the standard solution addition to the quartz capillary tube and on retention time constancy were performed for each of the tested standards. The relative standard deviation (R.S.D.) of the peak areas was in the 5–7%

range (5 replicate analysis) for 1,3,5-trimethoxybenzene and 1,3,5-tri-*tert.*-butylbenzene, whereas 1,2,4-benzenetricarboxylic acid trimethyl ester showed a rather high variation with an R.S.D. of about 50%, probably due to the non-ideal chromatographic behaviour of this compound.

The retention times of the tested compounds were very reproducible. Table 1 shows the results obtained after replicate injections on the same day (intra-day, 3 injections) and during 1 month (inter-day, 8 injections). Retention time R.S.D. for 1,2,4-benzenetricarboxylic acid trimethyl ester and 1,3,5-tri-*tert.*-butylbenzene appeared to be slightly greater in 1 month than in 1 day whilst 1,3,5-trimethoxybenzene retention time R.S.D. showed no variation.

Such data suggests that the selected compounds were not degraded by the pyrolysis conditions used in the present experiment. Rather, they vaporized in the hot Py–GC interface during the 3-min equilibration and focused on the top of the GC column. The vaporization of the compounds in these experimental conditions was confirmed by inserting the quartz capillary with the standards in the pyrolysis interface and performing the analysis without pyrolyzing it. The obtained areas were not significantly different from those obtained by pyrolyzing the same amount of internal standard. The manual insertion of the pyrolysis probe in the Py–GC interface was not a source of erratic results. However, the operator should rapidly close the interface after inserting the probe to avoid loss of internal standard.

A linear correlation (five points) between the amount of vaporized standard and the peak area was found for each of the compounds. The linear correlations are better represented by the following regression equations, where x represents the peak area ($P=0.05$): 1,3,5-tri-*tert.*-butylbenzene (ng pyrolyzed)$=1.4\cdot10^4(\pm2\cdot10^3)x$, $R^2=0.996$; 1,2,4-benzenetricarboxylic acid trimethyl ester (ng pyrolyzed)$=5\cdot10^3(\pm1\cdot10^3)x$, $R^2=0.986$; 1,3,5-trimethoxybenzene (ng pyrolyzed)$=5.5\cdot10^3(\pm1\cdot10^3)x$, $R^2=0.994$.

Each of the standards showed a very good linearity of response in the considered range but, due to the low reproducibility of 1,2,4-benzenetricarboxylic acid trimethyl ester, this compound was considered to be an unsatisfactory internal standard in Py–GC–MS analysis.

Both 1,3,5-tri-*tert.*-butylbenzene and 1,3,5-trimethoxybenzene were added to a sample of wheat straw to verify the possibility of coelution with lignocellulosic pyrolysis fragments. 1,3,5-Trimethoxybenzene had the same retention time of *cis*-isoeugenol, one of the common thermal degradation products of lignin, whereas the 1,3,5-tri-*tert.*-butylbenzene peak was in a zone of the pyrogram where no lignin pyrolysis fragment was present. Therefore, 1,3,5-tri-*tert.*-butylbenzene was used for the next applications.

Correction factors for the use of 1,3,5-tri-*tert.*-butylbenzene as internal standard were calculated by injecting 1 μl of a 0.2 mg/ml solution of phenolic standards (i.e. 2-methylphenol, guaiacol, 4-ethylphenol, 4-methylguaiacol, 2,6-dimethoxyphenol, eugenol, vanillin, acetovanillone, 4-allyl-2,6-dimethoxyphenol and syringaldehyde) and of 1,3,5-tri-*tert.*-butylbenzene in the GC–MS system (triplicate analysis). Results are reported in Table 2. With the exception of syringaldehyde, the average correction factor was 2.6, ranging from 1.94 for 4-allyl-2,6-dimethoxyphenol to 5.5 for vanillin. Both vanillin and syringaldehyde showed large standard deviations with respect to the other compounds. Syringaldehyde behaviour does not influence the analysis result

Table 1
Intra-day and inter-day retention time (t_R, scan) and repeatability (relative standard deviation, R.S.D) of tri-*tert.*-butylbenzene

Compound	Intra-day[a]		Inter-day[b]	
	t_R (scans)	R.S.D (%)	t_R (scans)	R.S.D (%)
1,3,5-tri-*tert.*-Butylbenzene	1579	0.04	1580	0.06
1,3,5-Trimethoxybenzene	1527	0.07	1528	0.07
1,2,5-Tribenzenecarboxylic acid trimethyl ester	2147	0.00	2147	0.04

[a] Three replicate analyses.
[b] Eight replicate analyses.

Table 2
Correction factors for some phenolic compounds calculated using 1,3,5-tri-*tert.*-butylbenzene as internal standard (triplicate analyses)

Compounds	Scan	Correction factors	R.S.D. (%)
2-Methylphenol	801	2.66	3.54
Guaiacol	863	2.43	3.61
4-Ethylphenol	1104	2.20	1.00
4-Methylguaiacol	1126	2.02	1.10
2,6-Dimethoxyphenol	1437	2.33	4.58
Eugenol	1441	2.03	2.80
Vanillin	1517	5.5	26.3
Acetovanillone	1658	2.06	11.9
4-Allyl-2,6-dimethoxyphenol	1828	1.94	6.34
Syringaldehyde	1910	22	50

because it is not one of the main compounds in the pyrograms of lignin [4]. Vanillin is one of the diagnostic compounds for lignocellulose analysis and its deviant behaviour could affect the data obtained by Py–GC–MS analysis.

In Fig. 1, the total ion chromatogram (TIC) of a wheat straw sample with an internal standard addition of 0.4 μg is shown. In Table 3, the identified lignin pyrolysis products and their absolute amounts (g/kg, mean value of five analyses), calculated using the internal standard method, are reported. The mean correction factor (i.e., 2.6) was used for pyrolysis fragments which were not in the pool of tested standard phenolic compounds.

The most abundant compound in the pyrogram was 4-vinylguaiacol (peak 9, 5.60 g/kg). Some other important pyrolysis fragments were 4-vinylphenol, 2,6-dimethoxyphenol, vanillin, and 4-vinyl-2,6-dimethoxyphenol (peaks 7, 10, 12 and 20). These phenolics are the product of the thermal cleavage at

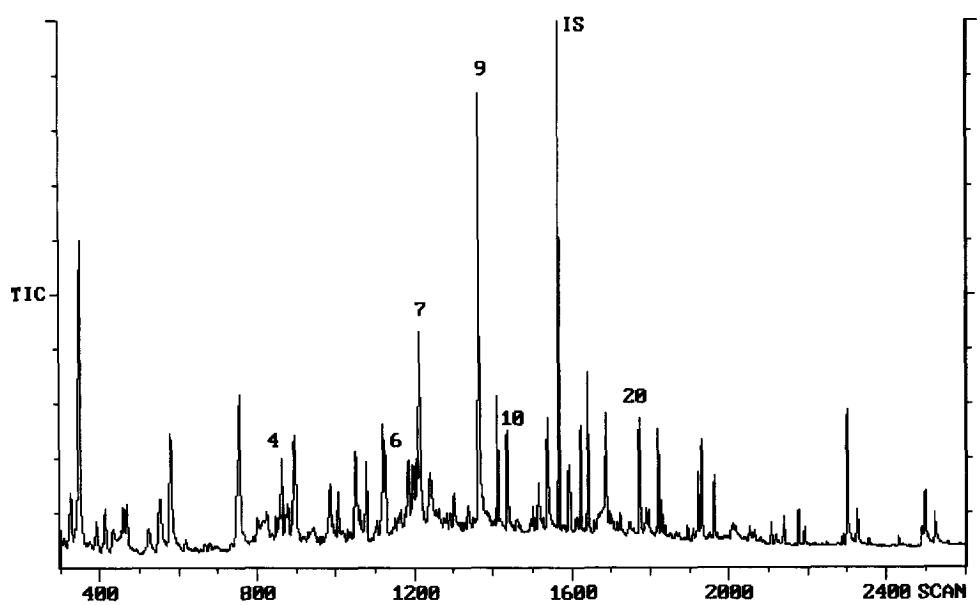

Fig. 1. Pyrogram of wheat straw (0.5 mg) with added internal standard (1,3,5-tri-*tert.*-butylbenzene, 0.4 μg). Numbers as in Table 3, I.S.=internal standard.

Table 3
Identified products from thermal degradation of lignin in a wheat straw sample and their absolute amounts (g/kg, mean of 5 replicate analyses) calculated using the correction factor value

No.	Compound	Scan	Mean
1	Phenol[a]	521	0.75
2	2-Methylphenol	801	0.15
3	4-Methylphenol[a]	852	0.30
4	Guaiacol	863	1.34
5	4-Ethylphenol	1104	0.11
6	4-Methylguaiacol	1126	1.33
7	4-Vinylphenol[a]	1213	2.36
8	4-Ethylguaiacol	1301	0.61
9	4-Vinylguaiacol[a]	1366	5.60
10	2,6-Dimethoxyphenol	1437	1.76
11	Eugenol	1441	0.06
12	Vanillin	1517	1.42
13	*cis*-Isoeugenol[a]	1527	0.08
14	2,6-Dimethoxy-4-methylphenol[a]+*trans*-Isoeugenol[a]	1594	1.48
15	Homovanillin[a]	1612	0.54
16	1-(4-Hydroxy-3-methoxyphenyl)propyne[a]	1630	Tr[b]
17	Acetovanillone	1658	0.02
18	4-Ethyl-2,6-dimethoxyphenol[a]	1716	0.04
19	Guaiacylacetone[a]	1724	0.37
20	4-Vinyl-2,6-dimethoxyphenol[a]	1772	1.81
21	4-Allyl-2,6-dimethoxyphenol	1828	0.33
22	4-Propyl-2,6-dimethoxyphenol[a]	1838	0.10
23	*cis*-4-Propenyl-2,6-dimethoxyphenol[a]	1895	0.19
24	Syringaldehyde	1910	1.14
25	*trans*-4-Propenyl-2,6-dimethoxyphenol[a]	1962	0.93
26	Acetosyringone[a]	2010	0.18
27	*trans*-Coniferyl alcohol[a]	2014	0.33
28	Syringylacetone[a]	2060	0.12
	Total		23.5
	R.S.D. (total) (%)		8.0

[a] A correction factor mean value (F_{mean}=2.6) was used for these compounds.
[b] Tr=Lower than 0.005.

different sites of the phenylpropanoid structure of lignin and are characteristic of lignocellulosic material pyrolysis patterns [4].

The total amount of phenolic compounds, related to the amount of lignin present in the pyrolyzed sample, was 23.5 g/kg. Pyrolysis yield for core lignin is lower than 20% in lignocellulosic samples [14]. Considering a pyrolysis yield of 20%, the total amount of lignin thermal degradation products in the wheat straw sample, as obtained using the internal standard method, was 117.5 g/kg. This value is consistent with bibliographic data obtained using more "classical" analytical techniques, such as neutral-detergent fiber analysis (NDF) [15] or Klason lignin [16], which are in the 100–200 g/kg range [17,18].

In conclusion, the internal standard method proved to be applicable for Py–GC–MS analysis of lignin, giving results comparable with data obtained using different analytical techniques. In this way, lignin removal or/and modification during chemical or biological treatment of cereal straw or other lignocellulosic materials can be satisfactorily analyzed by Py–GC–MS obtaining both quantitative and qualitative information. Considering that the lack of absolute quantitation is still one of the greatest

problem of Py–GC–MS, the availability of an on-line method, which allows to obtain the absolute quantitation of pyrolysis products, could be of great interest for researchers who work in the field of polymer analysis.

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