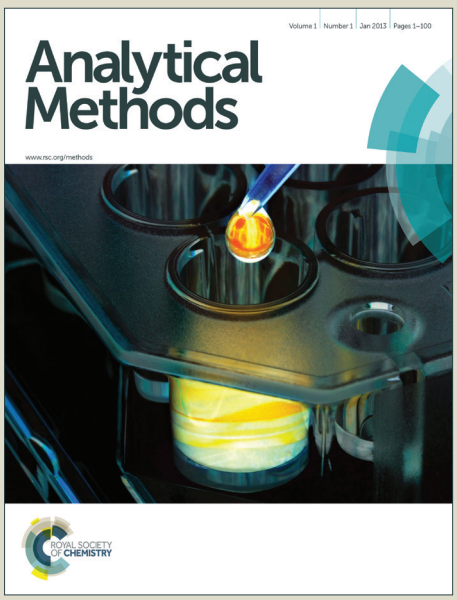
Analytical Methods

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/methods

Journal Name

ARTICLE

High throughput pyrogenic carbon (biochar) characterisation and quantification by liquid chromatography

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Wildson V. Cerqueira[a], Tatiana F. Rittl[b], Etelvino H. Novotny[c], Annibal D. Pereira Netto*[a]

Characterisation and quantification of the carbonaceous polyaromatic structure of pyrogenic carbon (PyC) are of paramount importance to evaluate the role of PyC in soil carbon sequestration. A new method of rapid resolution liquid chromatographic with UV diode array detection (RRLC-UV-DAD) was developed and is proposed to quantify benzene polycarboxylic acids (BPCA), which are considered molecular markers of the aromatic structure of PyC. The proportion of aromatic carbon structures and the degree of aromatic condensation obtained from different PyC, i.e. different biomass pyrolysed at final temperature (450°C) were quantified using the method of RRLC-UV-DAD. We showed that (i) the RRLC-UV-DAD analysis takes one quarter of the analysis time of conventional HPLC and half of the time of the GC analysis, increasing BPCA analysis throughput; (ii) the optimised method showed high overall precision and low limits of quantification and (iii) PyC produced from different tropical biomass exhibited a wide range of BPCA yields and patterns.

1.Introduction

Pyrogenic carbon (PyC), also known as black carbon, is the solid product of biomass pyrolysis that when deliberately applied to the soil, seeking for soil improvement and/or carbon sequestration, is denominated as biochar. The addition of PyC to soils is considered an alternative to increase the carbon stocks of soils because it can contribute to increase the most recalcitrant component of the soil organic matter.[1,2] The potential of PyC for carbon sequestration in soils may depend primarily on the concentration of polycondensed aromatic units,[3,4] because they provide a high recalcitrance (resistance to degradation) to PyC,[4,5] and greater carbon sequestration potential than soil organic matter.[6] The final concentration of polycondensed aromatic structures may differ[7] according to pyrolysis temperature and feedstock or biomass properties. PyC produced at high temperatures is richer in polycondensed units than PyC produced at low temperatures.[8,9] Although PyC can be produced from different biomass, the role of their characteristics, including plant structural carbon, to determine the concentration of polycondensed structures in PyC[7] is not clear. Therefore, the characterisation and quantification of polycondensed aromatic structures is important to assess the chemical quality of PyC, and infer about its persistence in the environment. However, the quantification of these structures represents an analytical challenge because PyC is not a compound with a defined chemical structure,[10] but rather a continuum of materials[11] with different proportions of polycondensed aromatic structures.

The characterisation and quantification of the typical polycondensed structures occurring in PyC can be performed through the determination of benzene polycarboxylic acids (BPCA) containing three to six carboxylic groups produced after chemical oxidation of PyC (Fig. 1). The BPCA produced in this way are considered specific molecular markers of polycondensed aromatic structures typically occurring in PyC[12,13] because the number of carboxylic acid groups present in each BPCA reflects the former degree of aromatic condensation of PyC. Therefore, the individual contributions of each BPCA can be used to evaluate the aromaticity (proportion of aromatic C) and polyaromatic condensation (size of aromatic C structure) of PyC.[8,14]

The BPCA method originally proposed[12] has been employed in the quantification of PyC in charcoals, soils and sediments over the past decade.[14–19] However, a study involving 17 laboratories revealed that the many steps need to clean up, transfer and derivatise the BPCA prior to quantification by gas chromatography (GC), may result in disparate results,[20,21] which varied up to 43 %, depending on the material analysed.

Dittmar (2008) determined PyC in seawater using high-performance liquid chromatography (HPLC) with diode array UV detection (HPLC-UV) to analyse BPCA, but the best BPCA separation took 90 min and the fastest one 60 min.[22] However, this analytical technique avoided the critical clean-up and derivatisation steps need for GC analysis.[12] This is an advantage of the HPLC-UV method because the potential risk of methodological artefacts is reduced.[22] Schneider et al (2011)[21] compared the efficiency of methods of GC with a flame ionisation detector (GC-FID) and HPLC with a photodiode array detector (DAD) in the determination of BPCA. They concluded the HPLC-DAD method is more robust for BPCA quantification than GC-FID.[21,22] In this way, rapid resolution liquid chromatography (RRLC) may be advantageous over HPLC,[23] reducing the analysis time and allowing a more sensitive quantification.[24]

[a] *Department of Analytical Chemistry and Post-Graduation Program in Chemistry , Institute of Chemistry, Federal Fluminense University 24020-141 Niterói, RJ, Brazil annibal@vm.uff.br*
[b] *Wageningen University, Dept. Soil Quality, 6700 AA Wageningen, Netherlands*
[c] *EMBRAPA Soils, 22460-000 Rio de Janeiro, RJ, Brazil*
WVC and TFR contributed equally to the work.

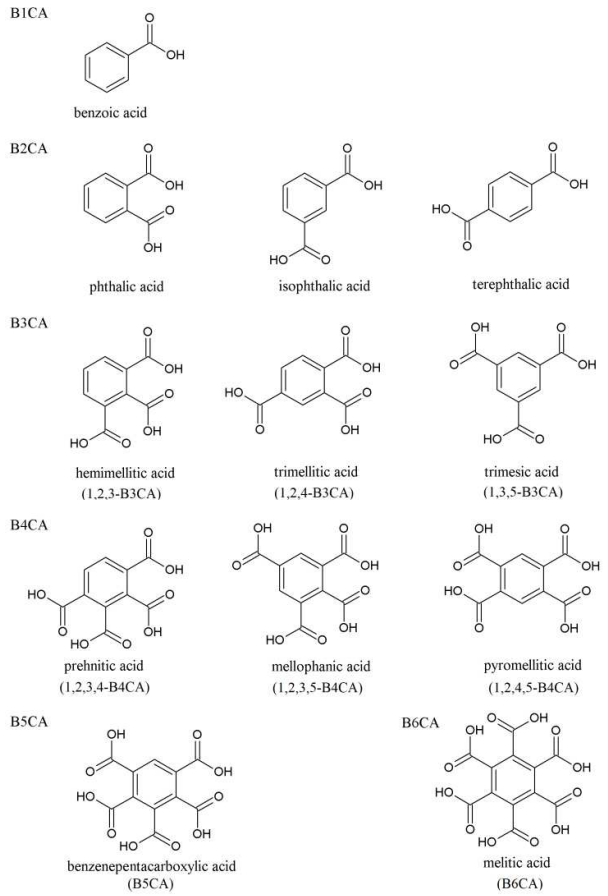


Fig. 1 Chemical structures, names and acronyms of all possible benzene polycarboxilic acids (BPCA).[12] BPCA containing three to six carboxylic groups were studied in the present work.

This study aimed to: (I) develop a new high-throughput method of BPCA quantification using RRLC-UV-DAD; and (II) present its application in the evaluation of the aromatic structure of PyC produced from different starting materials after pyrolysis at the same temperature.

2.Experimental

2.1. Standards and reagents

Tetrabutylammonium hydroxide, 2-propanol and methanol (HPLC grade, Tedia Brazil, RJ, Brazil), tris(hydroxymethyl) aminomethane hydrochloride (Tris-HCl, Sigma-Aldrich, MO, USA), and nitric acid, sodium acetate, sodium hydroxide and hydrochloric acid (all P.A. grade, Merck, RJ, Brazil) were used. Ultrapure water was prepared using a Simplicity System (Millipore, MA, USA) following reverse osmosis (Rios-DI, Millipore, MA, USA).

Solid BPCA (1,2,3-benzenetricarboxylic acid; 1,2,4-benzenetricarboxylic acid; 1,3,5-benzenetricarboxylic acid; 1,2,4,5-benzenetetracarboxylic acid; benzenepentacarboxylic acid and benzenehexacarboxylic acid) were purchased from Sigma-Aldrich (MO, USA) and used without further purification.

Standard stock solutions of individual BPCA (1000 mg L^{-1}) all prepared by dissolving 10 mg of each standard up to 10 mL of Tris-HCl (0.1 mol L^{-1} at pH 7.5). Standard solutions containing the six BPCA in concentrations of 0.5, 1, 5, 10, 20, 50, 80 and 100 mg L^{-1} were prepared by combining appropriate aliquots of each solution and diluting up to 1 mL using the mobile phase. Stock solutions were stored in amber glass vials at 2-4 °C in the dark and discarded one month after preparation, although they were stable for at least 3 months. Working standards were stable for at least one month, but they were discarded weekly.

2.2. Pyrogenic C samples and sample treatment

The samples of PyC were prepared from eucalyptus (*Eucalyptus dunnii* - DUN), pine (*Pinus taeda* - TAE), sugarcane bagasse (*Saccharum officinarum* - CAN), coconut (*Cocos nucifera* - COC), water hyacinth (*Eichhornia crassipes* - WAT) and candeia (*Vanillosmopsis erythropappa* - EXT), which was submitted to a previous treatment for bisabolol extraction. Samples of at least 100 g of PyC were powdered and homogenised prior to taking the aliquots used for oxidation and BPCA analysis. Details on pyrolysis parameters have been presented elsewhere[25]. In short, biomass was pyrolysed at a heating rate of 10 °C min^{-1} until reaching the final temperature of 450 °C. The samples were maintained at this temperature for 60 min.

2.3. Treatment of PyC samples for BPCA evaluation

Aliquots of 5 mg of pulverised PyC were weighed (0.01 mg precision) and transferred into 5 mL glass ampoules, which were sealed after addition of 0.5 mL concentrated HNO_3 (65% w/w). The ampoules were placed into microwave digestion vessels[22] and heated up to 170 °C for 8 h[12,13,22] using a laboratory oven. After oxidation, the ampoules were opened and the excess of nitric acid evaporated under a gentle N_2 flux. The samples were diluted up to 10 mL using the mobile phase (see below). Three to five aliquots of each PyC were independently oxidised and analysed.

2.4. Evaluation of UV-Vis absorption spectra

A UV-Visible spectrophotometer (Thermo Scientific Evolution 600, MA, USA) and a quartz cuvette (10 mm) were employed to obtain UV-Vis absorption spectra of solutions (10 mg L^{-1}) of individual BPCA. Solutions were prepared in water, in the mobile phase previously used for BPCA analysis (sodium acetate 0.1 mol L^{-1})[22] and in the mobile phase proposed in this study (Tris-HCl 0.1 mol L^{-1} at pH 7.5). The spectra obtained allowed selection of the best wavelengths for BPCA detection.

2.5. Chromatographic analysis

HPLC and RRLC separations were carried out using Agilent 1100 Series (CA, USA) and Agilent 1200 Series (CA, USA) chromatographic systems, respectively. Both consisted of a binary pump, a degasser, an automated injector, a thermostated column compartment and an UV-DAD detector, all controlled by Agilent ChemStations. Preliminary experiments showed that a detector slit of 4 nm and a response time of 0.1 min led to the best RRLC-UV-DAD detector responses and the use of the standard flow cell increased the intensity of the chromatographic signals.

A mobile phase composed of Tris-HCl (0.1 mol L^{-1}) + tetrabutylammonium hydroxide (2.5 mmol L^{-1}) (A) and a solution of the same composition prepared in water containing 2-propanol (70:30, v/v) (B) was employed for HPLC and RRLC separations.

2.5.1. HPLC-UV-DAD conditions

A Zorbax SB-C18 (150 x 2.1 mmx 3.5 µm; Agilent, CA, USA) column operated at 16 °C. A multiple step optimised gradient (Table 1) allowed the best separations under these conditions. A 10 µL volume was used for all analyses.

Table 1. Optimised gradient used for separation of BPCA using HPLC-UV-DAD

Time (min)	%A	%B	Flow rate (ml min^{-1})
0	99.0	1.0	0.3
3	99.0	1.0	0.3
10	98.6	1.4	0.3
20	97.9	2.1	0.3
40	96.7	3.3	0.4
50	96.1	3.9	0.3
51	99.0	1.0	0.3

2.5.2. RRLC-UV-DAD conditions

A Zorbax Eclipse Plus C18 column (50 x 4.6 mm, 1.8 µm; Agilent, CA, USA) was employed. A gradient was optimised and applied (Table 2) for the analysis of samples and standards. The column temperature was 20°C and the injection volume was 10 µL.

Table 2. Optimised gradient used for separation of BPCA using RRLC-UV-DAD

Time (min)	%A	%B	Flow rate (ml min^{-1})
0	94.0	6.0	2.1
10	86.0	14.0	2.1
12	0	100	0.5
16	94.0	6.0	2.1

2.6. BPCA identification and quantification

The determination of BPCA following PyC oxidation was carried out at 226 nm using the optimized RRLC-UV-DAD conditions. The analytes were identified by comparison of retention times of the individual BPCA, elution order and absorption spectra, which were usually simultaneously acquired during BPCA analysis. Simultaneous detection at 234 and 240 nm improved identification and allowed verification of interferences or coelutions.

Analytical curves were obtained after triplicate injections of standard solutions containing the six BPCA in concentrations ranging from 0.5 up to 100 mg L^{-1}. Linear fitting and curve parameters were obtained by the least squares method. The limits of detection (LD) and quantification (LQ) of individual BPCA were estimated by dividing respectively 3 and 10 times the signal-to-noise ratios by the angular coefficients of the analytical curves (IUPAC Criteria). Signal-to-noise ratios were estimated by the standard deviations of peak areas obtained after 7 subsequent injections of the 0.5 mg L^{-1} standard solution. The analytical curves were used to estimate the concentration of BPCA in the studied samples.

The precision of the entire method (oxidation of PyC and BPCA determination) was evaluated by oxidising 4 aliquots of 5 mg of the same PyC (sugarcane bagasse pyrolysed at 450°C) and analysing them under the optimised conditions. The analytical curve obtained for 1,2,4,5-benzenetetracarboxylic acid, the only commercially available isomer, was used for the quantification of the other benzenetetracarboxylic acids.[22] After quantification, the BPCA isomers containing three and four carboxylic groups were summed and quantified as ∑B3CA and ∑B4CA, respectively. This procedure was not used for B5CA and B6CA because they show no isomers.

2.7. Determination of ash content of the PyC samples

The ash content of the PyC samples was determined by calcination, after drying in a laboratory oven at 103° ± 2°C for 24h. The dryed samples were calcined at 750° ± 5°C for 6h in a muffle furnace. The ash content was calculated as the weight difference before and after calcinations.

3. Results and Discussion

3.1. Evaluation of absorption spectra in the analytical conditions

The pH of the mobile phase affects the dissociation constants of the BPCAs. At a pH around 8, all BPCA are completely dissociated, resulting in only one analytical form per compound. However, high pH values may damage most HPLC columns. The first step of the method development consisted of the evaluation of the UV-Vis spectra of BPCA solutions and the mobile phase (sodium acetate solution 0.1 mol L^{-1} with a pH around 8.8) previously employed for HPLC-UV analysis of BPCA.[22]

The mobile phase composed of sodium acetate 0.1 mol L^{-1} showed a strong absorbance in the lowest wavelength portion of the spectra (210 to 240 nm) (Fig. 2) preventing BPCA detection at wavelengths below 240 nm. Other mobile phases (*e.g.*, phosphate buffer) at the same pH were evaluated but most of them also showed significant absorption in that region of the spectra ($\lambda \leq 230$ nm). The mobile phase composed of Tris-HCl (0.1 mol L^{-1}) + tetrabutylammonium hydroxide (2.5 mmol L^{-1} at pH 7.5) was selected because its absorption was practically negligible at $\lambda \geq 226$ nm and it interfered little in the BPCA signals allowing (Fig. 2) the use of such wavelength in HPLC detection. Moreover, pairing effects that are beneficial to the separation resulted from using the tetrabutylammonium as the cation of the strong base used to buffer the mobile phase. Therefore, the wavelength of 226 nm and the mobile phase described above were selected for BPCA determination.

3.2. Optimisation of the RRLC method and evaluation of selected analytical features of the optimised method

The next stage of the study consisted of the evaluation and optimisation of the HPLC-UV separation, as described above. The optimized conditions allowed a complete separation of the BPCA within 50 min (Figure 3), 10 min faster than previous HPLC methods[21,22,26]. Large flow rates would possibly reduce analysis time but they would imply in larger pressures, which are limited by the maximum pressure (400 bar) of the conventional HPLC system. These results demonstrated the feasibility of the desired separation using this mobile phase, and indicated that use of RRLC could be a good option to continuing development to reduce further the analysis time.

The maximum recommended pressure for the RRLC system (600 bar) prevented the use of flow rates above 2.1 mL min^{-1}, which would possibly improve the separation quality. The addition of acetonitrile to the mobile phase B resulted in lower pressure, but also in poorer separations and coelution of 1,2,3-B3CA and 1,2,4-B3CA. The evaluation of other buffers at a similar pH (*e.g.*,

phosphate) resulted in worse BPCA separations. Moreover, they absorbed strongly in the detection region.

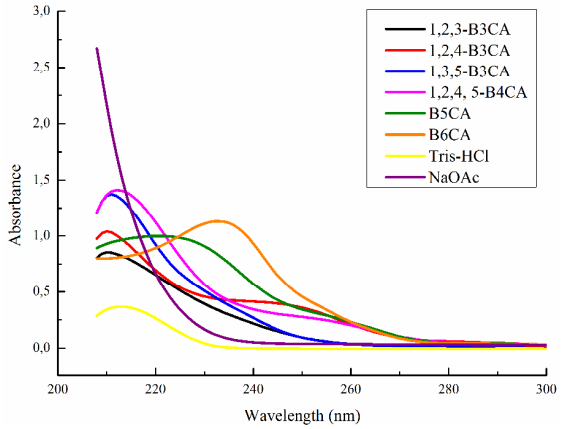


Fig. 2. Absorbance spectra of sodium acetate solution (0.1 mol L^{-1}), Tris-HCl solution (0.1 mol L^{-1} at pH 7.5) and individual BPCA (10 mg L^{-1}) in the mobile phase.

The optimised gradient and method employed allowed baseline separation of the BPCA (Fig. 4) within around 8 min. The chromatographic peaks obtained using RRLC were more symmetric and narrower than using HPLC. An additional period of 8 min was used to clean the chromatographic system and re-establish the initial chromatographic conditions, leading to a complete analysis in 16 min. The time needed for the separation of BPCA using conventional HPLC was previously considered a disadvantage when compared to GC.[21] However, the chromatographic method presented in this study was almost 4 times faster than previous HPLC-UV method,[22] and even faster than the GC method.[12] Even considering methods recently published, the chromatographic conditions presented here are comparatively advantageous with regard to the conditions used by Schneider et al. (2011),[21] Yarnes et al. (2011),[27] and Wiedemeier et al. (2013, 2015)[26,28], when the method throughput was considered.

The chromatographic areas of the BPCA were usually 10 to 20% larger at 226 nm than at 230 or 240 nm. A quantitative method was partly validated and employed for PyC evaluation. The analytical curves of the BPCA showed very good adherence to linear models and shown by the excellent correlation coefficients (>0.996) found in the studied range (0.5 to 500 mg L^{-1}) for all BPCA studied.

The analytical curves allowed for the estimation of LD and LQ. LD varied between 0.2 and 0.5 µmol L^{-1} whereas the LQ varied between 0.7 and 1.7 µmol L^{-1}. The LD values were similar to those previously found (0.2 to 0.5 µmol L^{-1})[22], demonstrating the applicability of the proposed method of BPCA determination. Considering the typical sample mass and dilution, LD varied between 0.4 and 1.0 µmol g^{-1} of PyC, whereas LQ varied from 1.3 to 3.3 µmol g^{-1} of PyC (Table 3).

The overall precision of the method was evaluated by the coefficients of variation (CV(%)) obtained after oxidation of four independent aliquots of PyC obtained from sugarcane bagasse pyrolysed at 450°C (Table 4).

The CV(%) were always below 2.5 % except in the case of 1,2,3-B3CA, possibly due to its relatively low concentration in the mixture and high comparative volatility. However, when the three sum of the B3CA acids (ΣB3CA) was considered, the CV(%) below 4% The overall precision was comparable or better than found elsewhere.[21] These values also represent estimates of the variation of our data.

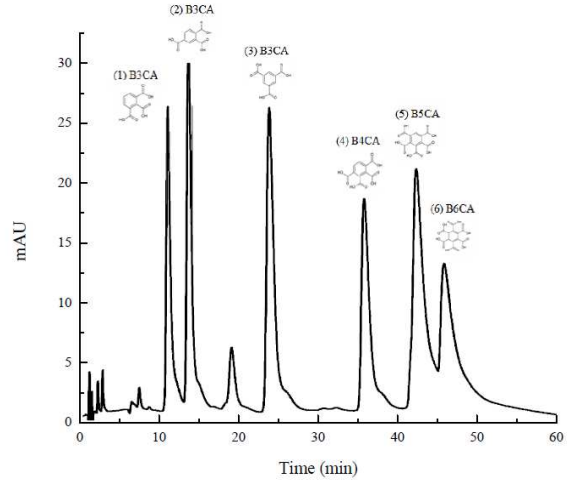


Fig. 3 Chromatogram of a solution containing all studied BPCA (10 mg L^{-1}) under optimised HPLC-UV-DAD conditions.

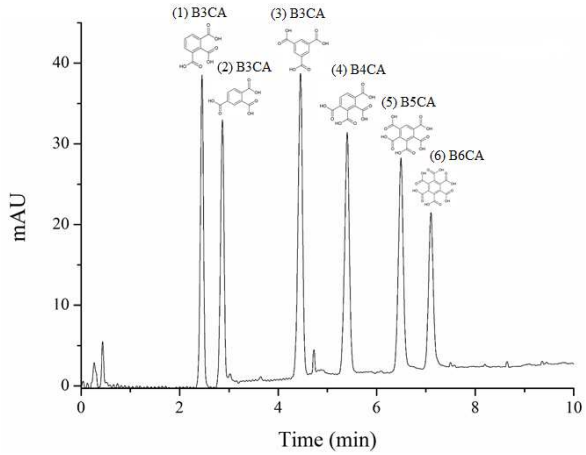


Fig. 4 Chromatogram of a solution containing all studied BPCA (10 mg L^{-1}) under optimised RRLC-UV-DAD conditions.

Table 3. Limits of detection and quantification of the individual BPCA obtained under optimised RRLC-UV-DAD conditions.

BPCA	Limits of detection (LD)[a]		Limits of quantification (LQ)[a]	
	µmol L^{-1}	mg g^{-1}	µmol L^{-1}	mg g^{-1}
1,2,3-B3CA	0.3	0.13	1.0	0.43
1,2,4-B3CA	0.4	0.17	1.3	0.56
1,3,5 B3CA	0.3	0.13	1.0	0.43
1,2,4,5-B4CA	0.4	0.20	1.3	0.66
B5CA	0.5	0.30	1.7	1.00
B6CA	0.2	0.14	0.7	0.47

[a]Considering the sample mass (0.5 mg) and final volume (1 mL), LD and LQ expressed as µmol g^{-1} correspond to twice the values expressed as µmol L^{-1}.

Table 4. Evaluation of the precision of the proposed method considering the BPCA produced by oxidation of PyC derived from sugarcane bagasse pyrolysed at 450°C

BPCA	BPCA concentrations (g kg^{-1} of PyC)[a]		CV(%)
	Means	Standard deviations	
1,2,3-B3CA	2.56	0.20	7.75
1,2,4-B3CA	6.66	0.14	2.01
1,3,5-B3CA	19.4	0.44	2.30
ΣB3CA[b]	28.7	1.10	3.85
1,2,4,5-B4CA	26.1	0.28	1.08
B5CA	69.9	0.60	0.85
B6CA	70.2	1.32	1.89

[a]n = 4

[b]considering the quantification as a sum of B3CA

3.3. Evaluation of aromaticity of PyC obtained from different biomass at the same pyrolysis temperature

PyC obtained from different biomass (DUN, TAE, CAN, COC, WAT and EXT) pyrolysed at the same temperature (450°C) were evaluated to study the effect of the biomass composition upon the BPCA patterns.

The BPCA method is non-stoichiometric, which means that all PyC is not converted to BPCA, because part of the carbon content of the sample is lost as CO_2. The application of a conversation factor (2.27) was recommended[12] to overcome this problem but it is based on the mean of BPCA contents found after analysis of activated and barbecue charcoal.[12] However, the conversion factor differs for other PyC[13] and the application of a single conversion factor for different PyC would introduce an additional error for quantification. Therefore, no correction factor was used in this study, but the BPCA concentrations were normalized to the mass of PyC oxidised.

Total BPCA concentration (ΣBPCA) varied from 127.5 g BPCA kg^{-1} PyC (WAT) to 288.8 g BPCA kg^{-1} PyC (TAE). Although some values were higher than those previously found for wood pyrolysed at 450°C (161.9 and 155.3 g BPCA kg^{-1} PyC),[21] the results showed the same order of magnitude and are therefore, comparable.

Different contributions of BPCA according to the number of carboxylic groups were observed. PyC obtained at 450 °C produced predominately B6CA and B5CA that corresponded to up to around 70% of ΣBPCA for TAE, EXT, COC and CAN. The proportions of B5CA/ΣBPCA were larger than that of B6CA/ΣBPCA for all PyC except for DUN that showed the largest proportion of ΣB4CA/ΣBPCA between the samples studied (Fig. 5).

Another key parameter in PyC studies is the degree of aromatic condensation[28,30] that can be accessed through the ratio B6PA/Total BPCA[31]. This ratio varied between the samples (Fig. 5): CAN showed the highest ratio whereas WAT showed the lowest ratio, indicating that the carbonization was the mildest for the sample containing the largest ash content (shown at the top of each column).

These differences of ΣBPCA production and their distribution is in some extent dependent on the ash content of each sample (Fig. 5), because at the same pyrolysis conditions, the carbonization efficiency depends on biomass composition and is more efficient with feedstock with lower ash content.[29]

Negative correlations of ash content and each BPCA group were always found (Table 5), with significant results with regard to B6CA, ΣBPCA and the ratio of B6CA/ΣBPCA.

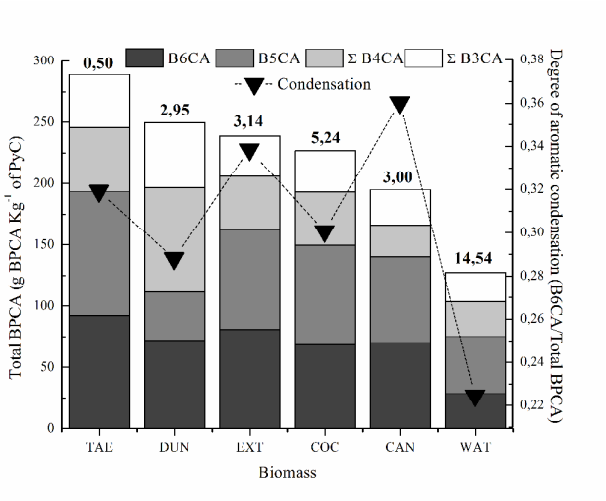


Fig. 5. Concentrations of BPCA (g BPCA Kg^{-1} of PyC; mean of 3 measurements) and degree of aromatic condensation in PyC prepared at 450 °C from different starting biomass: DUN (*Eucalyptus dunnii*), TAE (*Pinus taeda*), CAN (sugarcane bagasse - *Saccharum officinarum*), EXT (candeia - *Vanillosmopsis erythropappa*), COC (coconut - *Cocos nucifera*) and WAT (water hyacinth - *Eichhornia crassipes*). The numbers in the columns are the ash content.

Table 5 Correlations between each BPCA and ash content in the samples studied.

ΣB3CA	ΣB4CA	B5CA	B6CA	ΣBPCA	B6CA/ΣBPCA
-0.62	-0.44	-0.56	-0.97[a]	-0.90[a]	-0.83[a]

[a]Significant at p<5% (n = 6)

4.Conclusions

An improved, high-throughput, simple and novel method of RRLC-UV-DAD for the determination of BPCA in PyC, following nitric acid oxidation, was developed, validated and applied in this study. The method allowed BPCA determination with limits of quantification (LQ) and overall precision, expressed as coefficient of variation (CV(%)), similar or better than those of previous published methods, but its throughput was at least twice of that shown by these methods. Possibly the use of a pairing agent and the mobile composition (pH and buffer) were responsible for its optimum analytical performance.

Apart from this, the application of the analytical method to PyC prepared using tropical (Brazilian) biomass obtained at the same temperature and conditions (450 °C), showed that the composition of BPCA varied between the biomass and that it depended on the characteristics of the precursor biomass, especially ash content. The composition of PyC has environmental and agronomical interest because the recalcitrance and functions of PyC in soils are closely related to the aromaticity and degree of aromatic condensation.

With this respect, the new high throughput method of liquid chromatography presented here shows potential for application in routine analysis and characterization of PyC.

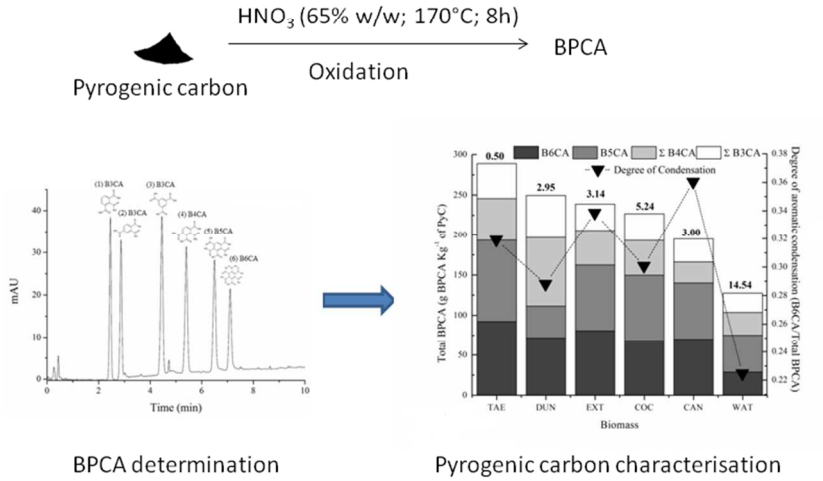
Acknowledgements

The authors thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação de Amparo à

Pesquisa do Estado do Rio de Janeiro (FAPERJ) for grants and fellowships. ADPN and EHN thank CNPq for individual research grants. All authors thank CMBF Maia and CFBV Alho for the samples of PyC.

References

1 B. Glaser, L. Haumaier, G. Guggenberger and W. Zech, *Naturwissenschaften*, 2001, **88**, 37-41.
2 J. Lehmann, *Nature*, 2007, **447**, 143-4.
3 N. Singh, S. Abiven, M. S. Torn and M. W. I. Schmidt, *Biogeosciences*, 2012, **9**, 2847–2857.
4 E. H. Novotny, M. H. B. Hayes, B. E. Madari, T. J. Bonagamba, E. R. De Azevedo, A. a. De Souza, G. Song, C. M. Nogueira and A. S. Mangrich, *J. Braz. Chem. Soc.*, 2009, **20**, 1003–1010.
5 B. P. Singh, A. L. Cowie and R. J. Smernik, *Environ. Sci. Technol.*, 2012, **46**, 11770–8.
6 C. Maia, B. Madari and E. Novotny, *Dyn. soil, Dyn. Plant*, 2011, **5**, 53–58.
7 A. V. McBeath, R. J. Smernik, E. S. Krull and J. Lehmann, *Biomass and Bioenergy*, 2014, **60**, 121–129.
8 M. P. W. Schneider, M. Hilf, U. F. Vogt and M. W. I. Schmidt, *Org. Geochem.*, 2010, **41**, 1082–1088.
9 A. V. McBeath, R. J. Smernik, M. P. W. Schneider, M. W. I. Schmidt and E. L. Plant, *Org. Geochem.*, 2011, **42**, 1194–1202.
10 C. A. Masiello, *Mar. Chem.*, 2004, **92**, 201–213.
11 C. Preston and M. Schmidt, *Biogeosciences*, 2006, **3**, 397–420.
12 B. Glaser, L. Haumaier, G. Guggenberger and W. Zech, *Org. Geochem.*, 1998, **29**, 811–819.
13 S. Brodowski, A. Rodionov, L. Haumaier, B. Glaser and W. Amelung, *Org. Geochem.*, 2005, **36**, 1299–1310.
14 K. Hammes, M. S. Torn, a. G. Lapenas and M. W. I. Schmidt, *Biogeosciences Discuss.*, 2008, **5**, 661–683.
15 B. Glaser, E. Balashov, L. Haumaier, G. Guggenberger and W. Zech, *Org. Geochem.*, 2000, **31**, 669–678.
16 C. I. Czimczik, C. M. Preston, M. W. I. Schmidt and E.-D. Schulze, *Global Biogeochem. Cycles*, 2003, **17**, 1020.
17 B. Glaser and W. Amelung, *Global Biogeochem. Cycles*, 2003, 17, 1064–1071.
18 A. Rodionov, W. Amelung, L. Haumaier, I. Urusevskaja and W. Zech, *J. Plant Nutr. Soil Sci.*, 2006, **169**, 363–369.
19 S. Brodowski, W. Amelung, L. Haumaier and W. Zech, *Geoderma*, 2007, **139**, 220–228.
20 K. Hammes, M. W. I. Schmidt, R. J. Smernik, L. A. Currie, W. P. Ball, T. H. Nguyen, P. Louchouarn, S. Houel, M. Elmquist, G. Cornelissen, J. O. Skjemstad, J. C. Dunn, P. G. Hatcher, W. C. Hockaday, D. M. Smith, C. Hartkopf-fro, A. Bo, P. M. Gschwend, D. X. Flores-cervantes, C. Largeau, C. Rumpel, G. Guggenberger, K. Kaiser, M. De Rosa, D. A. C. Manning and E. Lo, *Trial*, 2007, **21**.
21 M. P. W. Schneider, R. H. Smittenberg, T. Dittmar and M. W. I. Schmidt, *Org. Geochem.*, 2011, **42**, 275–282.
22 T. Dittmar, *Org. Geochem.*, 2008, **39**, 396–407.
23 J. R. Mazzeo, U. D. Neue, M. Kele, R. S. Plumb and W. Corp, *Anal. Chem.*, 2005, 460–467.
24 S. de M Ochs, M. Fasciotti, R. P. Barreto, N. G. de Figueiredo, F. C. Albuquerque, M. C. G. P. Massa, I. Gabardo and A. D. Pereira Netto, *Talanta*, 2010, **81**, 521–9.
25 C. F. B. V Alho, R. Auccaise, C. M. B. F. Maia and E. H. Novotny, in *Functions of Natural Organic Matter in Changing Environment*, eds. J. Xu, J. Wu and Y. He, Springer Netherlands, Dordrecht, 2013, pp. 1007–1011.
26 D. B. Wiedemeier, M. D. Hilf, R. H. Smittenberg, S. G. Haberle and M. W. I. Schmidt, *J. Chromatogr. A*, 2013, **1304**, 246–50.
27 D.B. Wiedemeier, S. Abiven, W.C. Hockaday, M. Keiluweit, M. Kleber, C.A. Masiello, A.V. McBeath, P.S. Nico, L.A. Pyle, M.P.W. Schneider, R.J. Smernik, G.L.B. Wiesenberg, M.W.I. Schmidt. *Org. Geochem*. 2015, **78**, 135-43.
28 C. Yarnes, F. Santos, N. Singh, S. Abiven, M. W. I. Schmidt and J. a. Bird, *Rapid Commun. Mass Spectrom.*, 2011, **25**, 3723–3731.
29 L. Yang, J. Ran, L. Zhang. *J. Energ. Resour.-ASME*, 2011, **133**, 1-7.
30 E.H. Novotny, C.M.B.F. Maia, M.T.M. Carvalho, B.E. Madari. *Rev. Bras. Ci. Solo.* 2015 (in press).



BPCA determination Pyrogenic carbon characterisation

254x190mm (96 x 96 DPI)