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Development of a method for fast analysis of phenolic molecular markers in biomass burning particles using high performance liquid chromatography/atmospheric pressure chemical ionisation mass spectrometry

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Abstract

A high performance liquid chromatography/atmospheric pressure chemical ionisation mass spectrometry (HPLC/APCI-MS) method for the fast analysis of 21 biomass burning tracers in particles samples has been developed. Separation was done with a Zorbax SB-C18 Rapid Resolution cartridge column (4.6 mm × 30 mm × 3.5 μm), using a $CH_3OH/H_2O/CH_3COOH$ gradient at a flow rate of 0.5 mL/min. The observed relative standard deviations (RSD) for the retention times and peak areas were <0.6 and <15%, respectively. With the short analytical column and the sensitive detector the total analysis time for the standard mixture was reduced to 15 min. Instrumental detection limits were <1 μM (S/N = 3) for all standard compounds except homovanillic acid (4.3 μM). The suitability of the developed method for the analysis of biomass burning particles is demonstrated by the measurements of five different real biomass burning samples. The results of these measurements showed clear differences between the different kinds of biomass and they are in good agreement with results from earlier studies in the literature.

Keywords: Biomass burning; HPLC/MS; Methoxyphenols; Nitrophenols; Phenolic compounds

1. Introduction

Combustion of biomass is an important source of organic particles and a variety of pollutants in the atmosphere. The organic matter of such particles is a mixture of different polar as well as non-polar compounds such as organic acids, terpenoids, sugars, alkanes, alkenes, polycyclic aromatic hydrocarbons (PAH) and phenolic compounds [1–4]. The phenolic fraction is typically only a small fraction (approximately 1–2%) of the total organic mass. However, possible health effects and their suitability as biomass burning tracers make the phenolic compounds interesting components of the particle mass [2,5–11]. In particular, the methoxy-substituted phenols are suitable as tracer compounds because they are both direct pyrolysis products of lignin (guaiacyl and syringyl compounds) as well as secondary oxidation products (e.g. vanillin). The concentrations and concentration ratios of these compounds are strongly influenced by the type of the combusted biomass and the combustion conditions. While softwood combustion produces mainly guaiacyl compounds (4-hydroxy-3-methoxyphenols), hardwood combustion forms more syringyl compounds (4-hydroxy-3,5-dimethoxyphenols) [1,2,7,8,10]. However, the chemical and photochemical stabilities and consequently the lifetime of these tracer compounds in the multiphase aerosol system are still not well characterised [1,2]. Since nitrogen oxides (NO_x) are released from biofuel combustion, also nitration products of the emitted phenolic fraction should be considered as tracer compounds [12,13]. Furthermore, theses nitro-compounds are also interesting in terms of human health and environmental pollution.

GC/MS has been commonly used for the determination of phenolic compounds in atmospheric particle samples [1,3,4,7–10,14,15]. This analytical method offers a couple of benefits for such applications, due to its selectivity, the high separation efficiency and the sensitive detection for a wide range of compounds. However, the GC analysis of polar and

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semi-volatile aromatic compounds normally requires a prior derivatisation step in order to improve the chromatographic separation and the detection sensitivity. This derivatisation step makes the sample preparation time-consuming. Good characterisation of recoveries is essential in order to achieve good quantification. Moreover, there is a risk of unwanted chemical reactions during the derivatisation process. Very recently, Simpson et al. [14] discussed problems of previous GC/MS methods for the analysis of methoxyphenols in ambient aerosol, due to the unexpectedly high chemical reactivity and the volatilisation of these compounds.

In the last years, an increasing number of researchers are adapting alternative methods to GC/MS such as HPLC/MS and CE/MS to the analysis of polar organic compounds in particle samples [16–20,29]. In particular the easy and fast sample pre-treatment, the short analytical time, the soft ionisation techniques and the simplicity of the method make the HPLC/MS coupling to an interesting tool for qualitative and quantitative routinely measurements. Numerous publications in the past reported the applicability of the HPLC for the separation of methoxy- and nitro-substituted phenols in various matrices [21–28].

The objective of the present study is to develop a fast and sensitive method for the analysis of phenolic compounds in ambient particles. In this work, the effect of the eluent composition, the pH and the column temperature was investigated in order to optimize the separation conditions and to fulfil the mass spectrometric requirements. The developed method was tested for the analysis of biomass burning particles from laboratory combustion of various biofuels.

2. Experimental

2.1. Chemicals

The purity of the standard compounds used in this work was better than 97% and were obtained from the following suppliers: ferulic acid, sinapic acid, syringealdehyde, vanillin, 4-nitrophenol, 2-nitrophenol, 2,6-dinitro-4-methylphenol, 2,4-dinitrophenol and 4-nitrocatechol from Fluka (Munich, Germany); vanillic acid, homovanillic acid, coniferyl aldehyde, 3,5-dimethoxy-4-hydroxyacetophenone, syringic acid, 4-hydroxycinnamic acid, 2,6-dimethyl-4-nitrophenol, 3-methyl-4-nitrophenol, 4-methyl-2-nitrophenol, 2-methyl-4-nitrophenol, 4,6-dinitro-2-methylphenol, 4-nitroguaiacol and vanillin-ring-$^{13}C_6$ from Sigma–Aldrich (Munich, Germany); 4-nitrophenol-ring-d_4, 2,4-dinitrophenol-ring-d_3 and 4,6-dinitro-2-methylphenol-ring-d_2 from Cambridge Isotope Laboratories (Andover, USA); methanol (HPLC grade) was purchased from Riedel de Haën (Seelze, Germany). Milli-Q (Millipore, Eschborn, Germany) grade water was used throughout this study.

The 10 methoxy- or hydroxy-substituted phenols in Table 1 were chosen as standard compounds, since they are commercially available and already classified as suitable biomass burning tracers [2,7,9,14,29]. In contrast to this the nitro containing analytes in Table 1 are to be tested on their suitability as marker substances. They were chosen after the following criteria: probability of formation by nitration processes, occurrence in the atmosphere and availability.

A stock solution (5 mM) with all 21 standard compounds (see Table 1) was prepared by dissolving them in methanol. The stock solution was stored in darkness at 4 °C. Calibration solutions (0.1, 1, 5, 10, 50, 100 μM) were prepared by diluting the stock solution.

2.2. Instrumentation

2.2.1. HPLC system

Liquid chromatographic separations were carried out on a HP 1100 liquid chromatography system (Agilent, Waldbronn, Germany). The HPLC system consists of a degasser unit (G1322A), a binary pump (G1312A), a DAD detector (G1315A) and a column oven (Spark Holland, Netherlands). Separations were carried out on a Zorbax SB-C18 Rapid Resolution cartridge column (4.6 mm × 30 mm, 3.5 μm particle, 80 Å, Agilent, Waldbronn, Germany). In order to protect the analytical column from microparticles a guard column (SecurityGuard, C8; 2.0 mm × 4.0 mm, Phenomenex, USA) was used. All separations were carried out using a gradient mode with a flow rate of 0.5 mL/min and an injection volume of 10 μL. During the gradient run the eluent composition was changed from water/methanol (90/10, v/v) to water/methanol (30/70, v/v)

Table 1
Molecular weight (M_r in g/mol) and detected ions (m/z) of standard compounds

Methoxy- or hydroxy-substituted phenols			Nitro-substituted phenols		
Compound	M_r	Detection	Compound	M_r	Detection
Vanillic acid	168	152	4-Nitrophenol	139	138
Homovanillic acid	182	136	2-Nitrophenol	139	138
Syringic acid	198	182	2,4-Dinitrophenol	184	183
Vanillin	152	136	Nitrocatechol	155	154
Syringaldehyde	182	166	4-Nitroguaiacol	169	153
3,5-Dimethoxy-4-hydroxy-acetophenone	196	165	2,6-Dinitro-4-methylphenol	198	197
4-Hydroxycinnamic acid	164	119	2,6-Dimethyl-4-nitrophenol	167	166
Sinapic acid	224	223	3-Methyl-4-nitrophenol	153	152
Ferulic acid	194	134	2-Methyl-4-nitrophenol	153	152
Coniferyl aldehyde	178	162	2-Methyl-4,6-dinitrophenol	198	197
			4-Methyl-2-nitrophenol	153	152