Journal of Chromatography A, 1218 (2011) 6369–6378

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Development of normal phase-high performance liquid chromatography-atmospherical pressure chemical ionization-mass spectrometry method for the study of 6,6′-bis-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-benzo [1,2,4]-triazin-3-yl)-[2,2′]-bipyridine hydrolytic degradation

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ARTICLE INFO

Article history:
Received 19 April 2011
Received in revised form 16 June 2011
Accepted 1 July 2011
Available online 8 July 2011

Keywords:
Normal phase chromatography
APCI-MS coupling
$CyMe_4BTBP$
Degradation products

ABSTRACT

In the field of nuclear waste management, the 6,6′-bis-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-benzo[1,2,4]-triazin-3-yl)-[2,2′]-bipyridine ($CyMe_4BTBP$) is a polycyclic N-based molecule eligible to remove actinides from spent nuclear fuel by liquid–liquid extraction processes. In such processes, the organic phase containing the extracting molecules will undergo hydrolysis and radiolysis, involving degradation products. The purpose of this work was to develop a normal phase chromatography (NP-HPLC) coupled to atmospherical pressure chemical ionisation-mass spectrometry (APCI-MS) method to separate and identify degradation products of $CyMe_4BTBP$ dissolved in octanol, submitted to HNO_3 hydrolysis. 1 mol L^{-1} HNO_3 hydrolysis conditions were used regarding the selective actinides extraction (SANEX) process, while 3 mol L^{-1} HNO_3 conditions were applied for the group actinide extraction (GANEX) process. The first step consisted in optimizing the chromatographic separation conditions using a diode array detection (DAD). Retention behavior of a non hydrolyzed mixture of N,N′-dimethyl-N,N′-dioctyl-hexyloxyethyl-malonamide (DMDOHEMA), a malonamide used in the SANEX process to increase the kinetic of extraction, and $CyMe_4BTBP$ were investigated on diol-, cyano-, and amino-bonded stationary phases using different mobile phase compositions. These latter were hexane with different polar modifiers, i.e. dioxane, isopropanol, ethanol and methylene chloride/methanol. The different retention processes in NP-HPLC were highlighted when using various stationary and mobile phases. The second step was the setting-up of the NP-HPLC-APCI-MS coupling and the use of the low-energy collision dissociation tandem mass spectrometry (CID-MS/MS) of the precursor protonated molecules that allowed the separation and the characterization of the main hydrolytic $CyMe_4BTBP$ degradation product under a 3 mol L^{-1} HNO_3 concentration. Investigation of the CID-MS/MS fragmentation pattern was used to suggest the potential ways leading to this hydrolysis degradation product. This NP-HPLC-APCI-MS method development is described for the first time for the $CyMe_4BTBP$ and should provide separation methods regarding the analysis of polycyclic N-based extracting molecules and more generally for the investigation of the organic phase coming from liquid–liquid extraction processes used in nuclear fuel reprocessing.

1. Introduction

In the framework of nuclear waste reprocessing, the well established plutonium uranium refining by extraction (PUREX) process allows the recovery of uranium and plutonium from spent nuclear fuel. The remaining waste however, still contains long-lived radioelements such as minor actinides (Np, Am, Cm) and long-lived fission products (I, Tc, Cs...), inducing significant long-term radiotoxicity. The partitioning of these elements and their transmutation in short-lived nuclides would significantly decrease the required storage time for nuclear waste [1]. In this field, one of the proposed schemes was two successive partitioning processes by liquid–liquid extraction technique [2]: the diamide extraction (DIAMEX) process aiming at co-separate the actinides (An(III)) and lanthanides (Ln(III)) from fission products, followed by the selective extraction of An(III) by the selective actinides extraction (SANEX) process. This second step represents a difficult challenge due to the similar chemical properties of An(III) and Ln(III), but their slightly different reactivity towards polydentate nitrogenated ligands [3–5], led to the design and

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doi:10.1016/j.chroma.2011.07.003

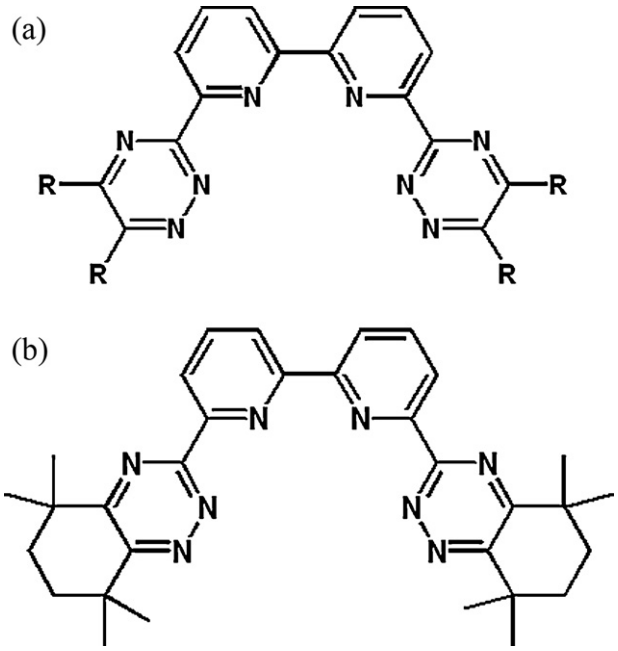


Fig. 1. (a) Structure of the 6,6′-bis-(5,6-dialkyl-[1,2,4]-triazin-3-yl)-[2,2′]-bipyridine (BTBP) derivatives. (b) Structure of the (6,6′-bis-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-benzo[1,2,4]-triazin-3-yl)-[2,2′]-bipyridine ($CyMe_4BTBP$).

development of a large number of polycyclic N-based extractants [6–8]. The BTBP family (6,6′-bis-(5,6-dialkyl-[1,2,4]-triazin-3-yl)-[2,2′]-bipyridine, Fig. 1(a)) [9–14] is particularly interesting as it combines high separation factors, good back extraction performance and improved resistance to hydrolysis and radiolysis, particularly for the $CyMe_4BTBP$ (6,6′-bis-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-benzo[1,2,4]-triazin-3-yl)-[2,2′]-bipyridine, Fig. 1(b)) [15,16]. Due to these features, $CyMe_4BTBP$ has also been considered for the group actinide extraction (GANEX) process [17], in order to simultaneously extract all the actinides from the dissolved spent fuel for transmutation purposes [18]. The GANEX process takes place in highly acidic medium (nitric acid in the aqueous phase between 3 and 6 mol L^{-1}), whereas the SANEX process operates with a 1 mol L^{-1} HNO_3 aqueous phase.

Although the $CyMe_4BTBP$ seems to exhibit several required characteristics regarding the processes conditions, the organic phase will be inevitably submitted to hydrolysis and radiolysis through the recurring extraction cycles, generating degradation products. Until now, studies dealing with the $CyMe_4BTBP$ degradation have been limited to the extraction performances evaluation after several extraction cycles using different organic diluents in contact with 1 mol L^{-1} HNO_3 aqueous phase [15,16,19]. To the best of our knowledge, $CyMe_4BTBP$ degradation products have never been identified nor characterised, which is critical to evaluate their impact on the extraction processes performance.

Electrospray-mass spectrometry (ES-MS) is a valuable technique which has been used for diverse extracting molecules degradation products identification, but without prior separation step [20–23]. High performance liquid chromatography (HPLC) coupled with mass spectrometry (MS) is powerful for the separation and characterization of organic molecules, but its application remains scarce regarding the analysis of extracting molecules used in the nuclear cycle [24,25]. Reversed phase (RP) HPLC is extensively and traditionally used for the separation of various compounds due to the availability of a wide variety of stationary phases and their selectivity. However, limited solubility of certain organic compounds in aqueous mobile phases and analysis of polar compounds

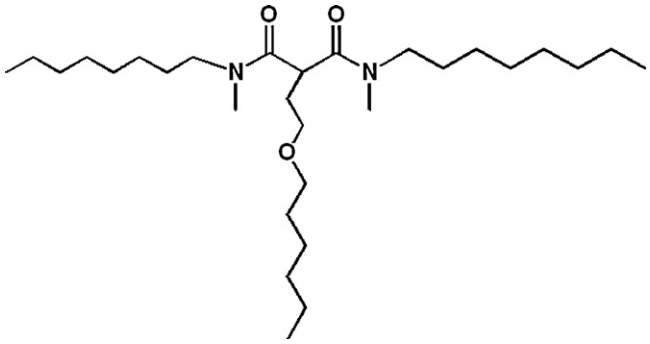


Fig. 2. Structure of the N,N′-dimethyl-N,N′-dioctyl-hexyloxyethyl-malonamide (DMDOHEMA).

represent some drawbacks to this technique. Normal phase-high performance liquid chromatography (NP-HPLC), involving polar bonded stationary phases, becomes then an alternative approach [26]. Moreover, NP-HPLC does not require the use of salts in the eluents, which is a great advantage regarding the coupling with mass spectrometry. Applications of NP-HPLC to separate polar compounds of different acid–base properties, e.g. amines, synthetic intermediates, pharmaceutical drugs, chiral and biologically active molecules, organic mixtures from crude oils are reported in the literature [27–37].

In this work, we report the development of a NP-HPLC hyphenated with atmospheric pressure chemical ionisation-mass spectrometry (APCI-MS) method, in order to characterize $CyMe_4BTBP$ hydrolytic degradation products produced in octanol diluent under 1 and 3 mol L^{-1} HNO_3. In a first step, the separation method has been developed and optimized using several stationary and mobile phases coupled to a diode array detector (DAD). As a second step, separation and identification of the main $CyMe_4BTBP$ hydrolytic degradation product have been investigated by NP-HPLC-APCI-MS using the optimized separation conditions. This analytical method can be ultimately expanded to the analysis of $CyMe_4BTBP$ degradation products produced by both hydrolysis and radiolysis in the SANEX or GANEX extraction processes conditions, which should induce more complex degradation pathways.

2. Experimental

Octanol/nitric acid system was used regarding the SANEX and GANEX liquid–liquid extraction processes. In both processes, $CyMe_4BTBP$ is the extracting molecule contained in the octanol phase, whereas HNO_3 concentration was 1 mol L^{-1} for SANEX and 3 mol L^{-1} for GANEX respectively. N,N′-dimethyl-N,N′-dioctyl-hexyloxyethyl-malonamide (DMDOHEMA, Fig. 2) is a malonamide that was used in the SANEX process in order to increase the kinetic of extraction.

2.1. Reagents and solvents

The 6,6′-bis-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-benzo[1,2,4]-triazin-3-yl)-[2,2′]-bipyridine ($CyMe_4BTBP$) and the 2,6-bis(5,6-isopropyl-1,2,4-triazin-3-yl)-pyridine (iPrBTP) were provided by the CEA/DEN/DRCP/LCSE (Laboratoire de Chimie des Systèmes Extractants, Marcoule, France). N,N′-dimethyl-N,N′-dioctyl-hexyloxyethyl-malonamide (DMDOHEMA) was synthesised in the CEA/DEN/DPC/LSRM (Laboratoire de Spéciation des Radionucléides et des Molécules, Saclay, France). All solvents used (hexane, isopropanol, ethanol, dioxane, methanol and methylene chloride) were HPLC grade. Nitric acid NORMAPUR 65% and $NaNO_2$ were purchased from Fisher (Fisher Scientific, UK).

2.2. Instruments

A chromatographic system composed of a binary pump HPLC 525 (Biotek Instruments, Germany), a degasser DEGASYS DG1210 (Uniflows), a column oven, an autosampler Smartline Autosampler 3900 (Knauer, Germany) and a diode array detector PDA S2800 (flow cell 10 μL) (Knauer, Germany) was used.

The experiments were carried out with two NH_2 columns, Nucleosil NH_2 250 × 4.6 mm (Macherey Nagel, Germany) and Luna NH_2 150 × 4.6 mm (Phenomenex, USA); two CN columns, Discovery Cyano 250 × 4.6 mm (Supelco, USA) and Luna CN 150 × 4.6 mm (Phenomenex, USA); and two diol columns, Inertsil Diol 250 × 3 mm (GL Sciences, Japan) and LiChrospher Diol 250 × 4 mm (Agilent Technologies, Germany). All columns were packed with 5 μm particles. The flow rate was 1, 0.4 and 0.33 mL/min for 4.6, 3 and 2 mm i.d. columns respectively. Temperature was set to 30 °C. 10 μL of the samples were injected for analysis. $CyMe_4BTBP$ displayed an absorption spectrum with maximum at 230 nm, 290 nm and a shoulder at 250 nm whereas a peak was observed at 204 nm for DMDOHEMA. The following wavelengths were selected for the analysis: λ = 200, 230, 250 and 290 nm.

2.3. NP-HPLC analyses

2.3.1. Optimization of the chromatographic method using a DAD

Three stock solutions of non hydrolysed molecules: $CyMe_4BTBP$, DMDOHEMA and $CyMe_4BTBP$ + DMDOHEMA were prepared in octanol at 10^{-2} mol L^{-1}. Samples were prepared by dilution of the corresponding stock solutions in ethyl acetate or ethanol to obtain the analytes at 10^{-4} mol L^{-1}. For each column (amino, diol and cyano), different compositions of mobile phase were tested in order to determine the best combination of stationary/mobile phases for the separation of $CyMe_4BTBP$ and DMDOHEMA. The mobile phases were done by hexane and a small percentage of polar solvent (i-PrOH, Dx, EtOH and CH_2Cl_2/MeOH).

2.3.2. Hydrolysis experiments

The hydrolysis of the $CyMe_4BTBP$ contained in the organic phase of the octanol/nitric acid (1 and 3 mol L^{-1} HNO_3) system was further monitored using the optimized chromatographic method. The organic phase made of 10^{-2} mol L^{-1} $CyMe_4BTBP$ in octanol and the 1 mol L^{-1} HNO_3 aqueous phase containing 5×10^{-2} mol L^{-1} $NaNO_2$, were contacted in a round-bottom flask equipped with a condenser and heated at 60 °C. $NaNO_2$ was added to mimic radiolytic products stemming from HNO_3 radiolysis [38,39]. The organic phase was sampled over time and each sample was centrifuged. 10 μL of the organic phase were then taken and diluted in ethanol or ethyl acetate to obtain a 10^{-4} mol L^{-1} concentration before NP-HPLC-DAD analysis. The same experiment was carried out with a 3 mol L^{-1} HNO_3 aqueous phase containing 15×10^{-2} mol L^{-1} $NaNO_2$. Samples of the organic phase were analysed with the Discovery CN 250 × 4.6 mm column in hexane/ethanol 85/15 + 0.1% DEA condition. Hence, the monitoring of the $CyMe_4BTBP$ hydrolysis has been performed by NP-HPLC-DAD and analysis of the main hydrolytic degradation has been further carried out by NP-HPLC-APCI-MS, which experimental conditions are described in the next section.

2.4. NP-HPLC-APCI-MS

An LCQ Advantage (Thermo Finnigan, USA) mass spectrometer with an APCI source was used. LC-MS experiments were carried out with a Surveyor (autosampler, pump and diode array detector) system (Thermo Finnigan, USA). The optimization of the mass spectrometer parameters was carried out under LC conditions. Through a zero dead volume Tee, were directed into the mass spectrometer:

- the mobile phase (hexane/ethanol 85/15 + 0.1% of DEA) at the optimized flow rate, without column
- the sample prepared in ethanol at 10^{-4} mol L^{-1} through a syringe pump.

The optimized mass spectrometer parameters were: vaporisation temperature 450 °C, sheath gas flow rate 80 L/min, auxiliary gas flow rate 24 L/min, Corona discharge 5 μA, capillary temperature 200 °C and capillary tension 33 V. These parameters were further used for NP-HPLC-APCI-MS analyses.

3. Results and discussion

In liquid–liquid extraction processes, the organic phase is a solution of the extracting molecule in a diluent (octanol in our work), whereas the aqueous phase contains the elements to remove in nitric acid. In this work, the nitric acid concentrations in the aqueous phase were 1 and 3 mol L^{-1} regarding the SANEX and GANEX processes respectively. For the SANEX process, a diamide modifier, the DMDOHEMA (Fig. 2), was added to the organic phase in order to increase the kinetic of extraction. Prior to the LC-MS experiments, the chromatographic separation method has been developed with a DAD and aimed at eluting the DMDOHEMA in the injection peak, to avoid any perturbations caused by its degradation products.

3.1. Chromatographic separation method development

Normal phase liquid chromatography was chosen in order to work in 100% organic medium, as organic phase diluent is octanol, non miscible with water. This feature prevents the use of RP-HPLC aqueous mobile phases. The basic retention mechanism in NP-HPLC is based on competitive adsorption between the polar constituent of the mobile phase (the modifier) and the solute for polar active sites of the stationary phase [40]. Normal phase processes of retention are sensitive to hydrogen bonding and dipole–dipole interactions [41]. Residual silanols of the bonded phase must be considered as additional active sites for retention and play a particular important role in retention and selectivity of basic compounds with cyano phases [42]. According to the nature of the solutes and accessibility of residual silanols, interactions with these latter induce more or less peak tailing and sometimes irreversible adsorption. The use of basic additives in the mobile phase is widespread to block residual silanols and therefore overcoming these drawbacks [43,44]. It must be pointed out that basic additives can also be used with amino and diol phases to improve peak shape, but not as widely as with cyano phases. Since the chemical nature of the stationary phase can have a major effect on selectivity, amino, diol and cyano bonded stationary phases were tested in this work.

The mobile phase was a mixture of a non-polar solvent (hexane) and a polar solvent, the modifier (isopropanol (i-PrOH), ethanol (EtOH), dioxane (Dx) and methylene chloride/methanol (CH_2Cl_2/MeOH)). The various modifier percentages provide a wide range of solvent strengths mobile phases, governing the solutes retention time. This should allow the separation of molecules of quite different polarity such as DMDOHEMA and $CyMe_4BTBP$. The solvent selectivity, i.e. proton donor/acceptor and dipole characteristics have also to be considered when describing retention mechanisms. However, solutes retention cannot be described apart from solvent strength and selectivity, since additional phenomena take often place. Localization, reflecting the tendency of polar molecules (solute or modifier) to strongly interact with adsorbing active sites such as residual silanols and bonded phase sites, as

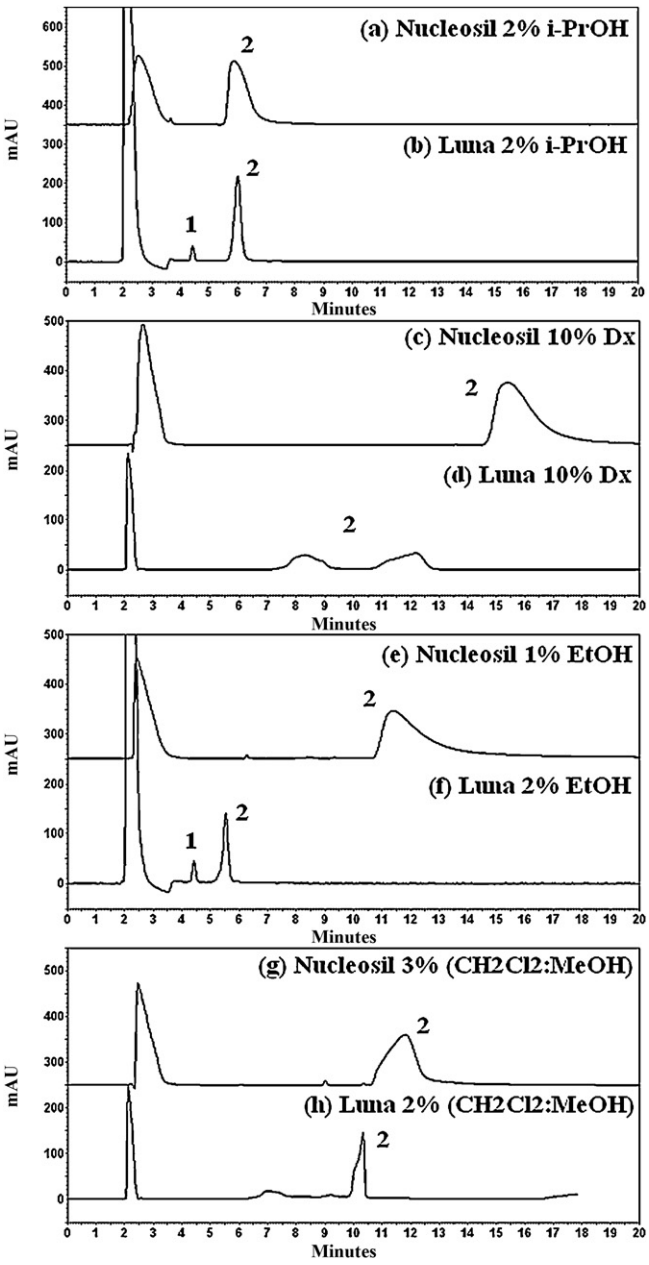


Fig. 3. (a–h) Effect of the modifier on the retention of CyMe4BTBP and DMDOHEMA using two amino phases (Nucleosil 250 × 4.6 mm (Macherey Nagel) and Luna 150 × 4.6 mm (Phenomenex)) in isocratic eluting conditions. Mobile phase: hexane as non polar solvent containing low percentage of polar modifiers (dioxane (Dx), isopropanol (i-PrOH), ethanol (EtOH) and methylene chloride/methanol (CH_2Cl_2/MeOH)). **1** and **2** are for DMDOHEMA (10^{-4} mol L^{-1}) and $CyMe_4BTBP$ (10^{-4} mol L^{-1}) respectively. Detection at the absorption maximum for **1** and **2**.

well as the secondary solvent effects, resulting from solute–solvent interactions in the adsorbed and non-adsorbed mobile phase, give rise to some changes in retention and selectivity [45].

3.1.1. Amino phases

Amino groups seem to be the primary adsorption sites for solutes, as reported by several authors [46,37,47]. Their basic feature should induce preferential interaction with acidic compounds.

Although DMDOHEMA (compound 1) eluted in the injection peak as required, $CyMe_4BTBP$ (compound 2) had a poor peak shape with tailing with the whole mobile phases, using the Nucleosil column (Fig. 3(a), (c), (e) and (g)). This tailing is characteristic of interactions with residual silanols, suggesting stronger polar interactions with these latter for the Nucleosil column compared to the Luna one. For same polar stationary phases coming from different manufacturers, substantial variability in the concentration of surface silanol groups could occur. Therefore, polarity and hydrogen bonding properties of the stationary phase will be affected, involving different retention phenomena for two columns. Although residual silanols amount is not available for the both phases, we can reasonably suggest that these latter are in higher concentration for the Nucleosil column.

With the Luna column, DMDOHEMA and $CyMe_4BTBP$ were separated with good selectivity and resolution, using i-PrOH and EtOH as modifiers (Fig. 3(b) and (f)). EtOH and i-PrOH are localizing solvents, able to interact with the polar sites of the stationary phase (i.e. polar bonded amino groups and residual silanols) principally through hydrogen binding. The retention of solutes able to interact with the active bonded sites should therefore be enabled by competition. However $CyMe_4BTBP$ exhibits basic properties that are not complementary to the amino bonded phase, therefore $CyMe_4BTBP$ was involved in weak interactions with the amino bonded groups, leading to weak retention of this latter as observed on Fig. 3(b) and (f). Small percentage of modifier was necessary (2%) to achieve a separation of DMDOHEMA (R_t = 4.5 min) and $CyMe_4BTBP$ (R_t = 6 min), confirming that $CyMe_4BTBP$ did not specifically interact with the active sites of the amino phase. In general, weak polar solutes are weakly retained or not retained at all. DMDOHEMA (Fig. 2), notably less polar than $CyMe_4BTBP$ due to its octyl lateral chains, was not significantly less retained, confirming again the previous observation and the reduced selectivity of these combined stationary and mobile phases toward $CyMe_4BTBP$.

Dioxane is also a localizing modifier but less polar than i-PrOH and EtOH, which can partly explain that higher dioxane percentage was needed to elute $CyMe_4BTBP$. Since $CyMe_4BTBP$ and dioxane exhibit basic and polar properties, they should specifically compete for active sites of a polar bonded phase. These properties are not complementary to those of the basic NH_2 bonded stationary phase; therefore no retention phenomenon should occur. However, we must keep in mind that residual silanols are additional adsorption sites for solute. In our case, it seemed that the major interactions took place with residual silanols, as revealed by the observation of broad tailing peaks (Fig. 3(d)).

2% of (CH_2Cl_2/MeOH) mixture led to more retained $CyMe_4BTBP$ compared to EtOH and i-PrOH modifiers and DMDOHEMA elution in the injection peak (Fig. 3(h)). CH_2Cl_2 is a non localizing modifier and as observed in our work, the use of localizing and non-localizing modifier can change the mobile phase selectivity. In this latter case, more complex retention mechanisms, involving secondary solvent effects, such as $CyMe_4BTBP$ interaction with the adsorbed modifier, might occur and explain the stronger $CyMe_4BTBP$ retention. In addition, Salotto et al. [48] found that with CH_2Cl_2 as modifier, amino phases preferentially retain basic solute despite their non complementary properties toward the stationary phase.

3.1.2. Diol phases

Diol stationary phases could be considered as deactivated silica since hydroxyl groups linked to carbon atoms are weaker proton donors. Bonded hydroxyl groups should be involved in hydrogen interactions with proton acceptor molecules. Due to the polar and basic properties of $CyMe_4BTBP$, stronger and more specific interactions should be reasonably expected with diol than with amino phases. Nevertheless, these interactions were not excessively stronger since slightly higher percentage of polar modifier (5%) was needed to give $CyMe_4BTBP$ retention times in the same range than the amino phases (Fig. 4(a), (b), (c), (d), (e), (f) and (g)). Interaction mechanisms more complex than hydrogen bonding, like secondary solvent effects, might occur for $CyMe_4BTBP$ retention on diol stationary phases.

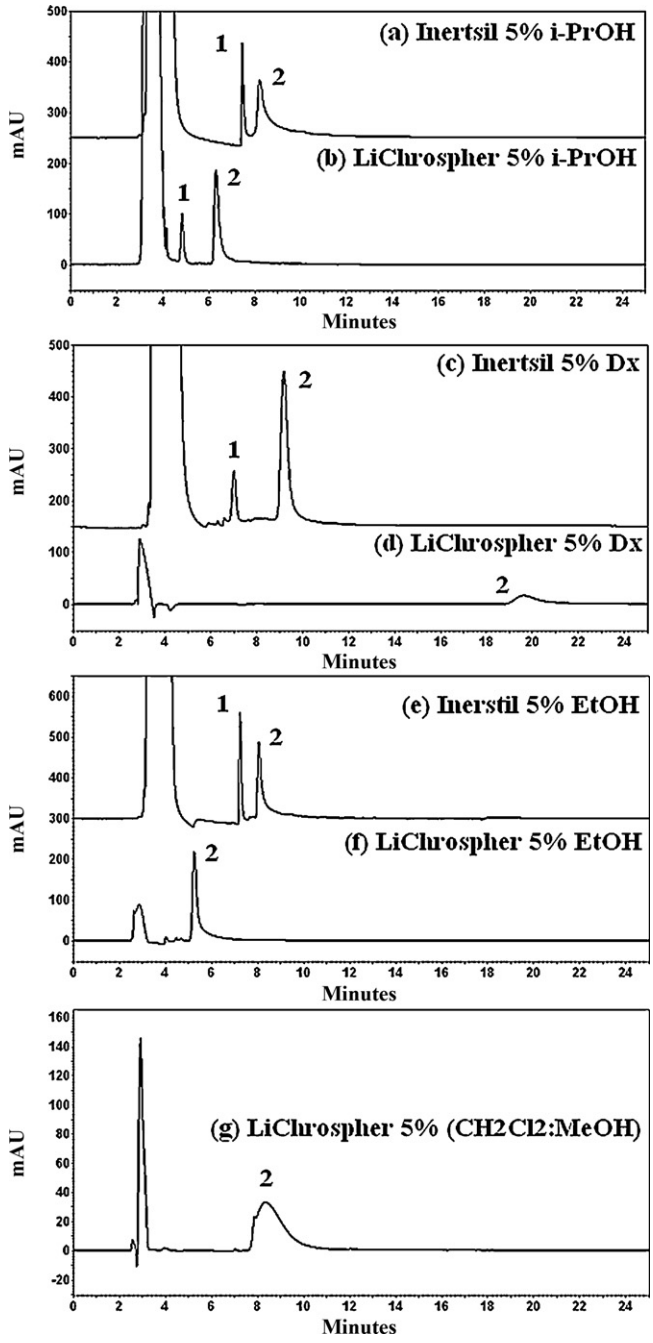


Fig. 4. (a–g) Effect of the modifier on the retention of CyMe4BTBP and DMDOHEMA using two diol phases (Inertsil 250 × 3 mm (GL Science) and LiChrospher 250 × 4 mm (Agilent)) in isocratic eluting conditions. Mobile phase: hexane as non polar solvent containing low percentage of polar modifiers (dioxane (Dx), isopropanol (i-PrOH), ethanol (EtOH) and methylene chloride/methanol (CH_2Cl_2/MeOH)). **1** and **2** are for DMDOHEMA (10^{-4} mol L^{-1}) and CyMe4BTBP (10^{-4} mol L^{-1}) respectively. Detection at the absorption maximum for **1** and **2**.

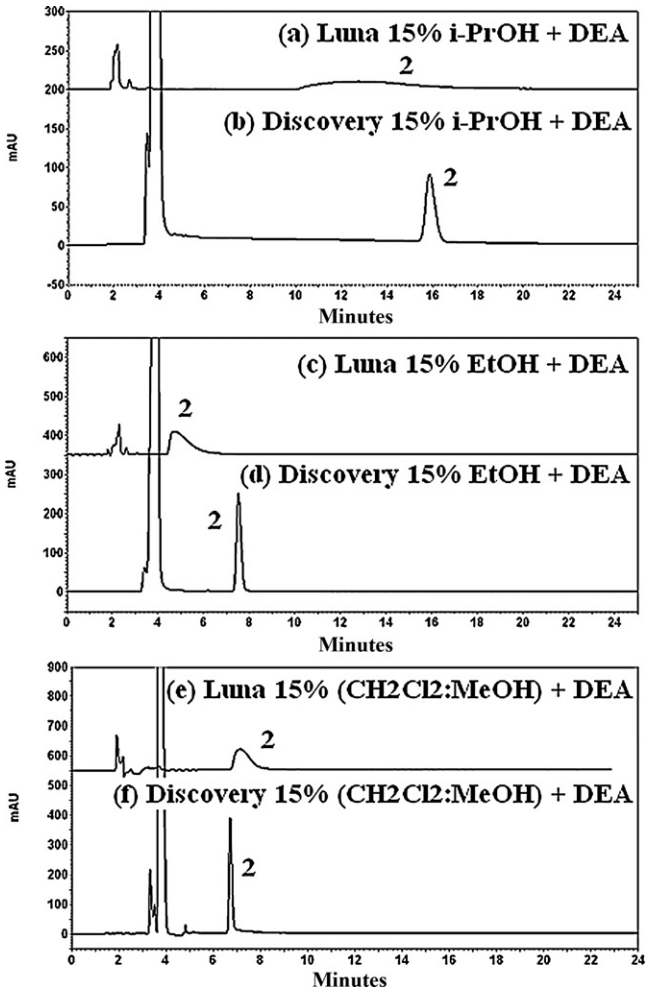


Fig. 5. (a–f) Effect of the modifier on the retention of CyMe4BTBP and DMDOHEMA using two cyano phases (Luna 150 × 4.6 mm (Phenomenex) and Discovery 250 × 4.6 mm (Supelco)) in isocratic eluting conditions. Mobile phase: hexane as non polar solvent containing low percentage of polar modifiers (dioxane (Dx), isopropanol (i-PrOH), ethanol (EtOH) and methylene chloride/methanol (CH_2Cl_2/MeOH)). **1** and **2** are for DMDOHEMA (10^{-4} mol L^{-1}) and CyMe4BTBP (10^{-4} mol L^{-1}) respectively. Detection at the absorption maximum for **1** and **2**.

EtOH and i-PrOH, as polar localizing modifiers, should interact with the bonded hydroxyl groups and the residual silanols, to allow the solutes retention by competition. Although the Lichrospher column led to enhanced selectivity and resolution with respect to the Inertsil one, this stationary-mobile phases combination was not very selective of the $CyMe_4BTBP$ (Fig. 4(a), (b), (e) and (f)). As for the amino phases, DMDOHEMA was not extensively less retained than $CyMe_4BTBP$. In all cases, peak tailings were assigned to interactions with residual silanols. We can notice that better resolution and selectivity were obtained for the separation of $CyMe_4BTBP$ and DMDOHEMA using the Inertsil column and dioxane modifier (Fig. 4(c)). As polar and basic compounds, dioxane and $CyMe_4BTBP$ exhibit complementary properties to compete for the active sites of the stationary phase. On the other hand, DMDOHEMA eluted in the injection peak and $CyMe_4BTBP$ eluted as a broad peak at around 20 min with the Lichrospher column (Fig. 4(d)). This behavior can reasonably be rationalised assuming that residual silanols were predominantly involved in retention. This emphasises again that same packed stationary phases coming from different manufacturers can lead to different chromatographic performances. (CH_2Cl_2/MeOH) was not a well adapted modifier for the Inertsil column since conditions were not met to observe retention. With the Lichrospher column, $CyMe_4BTBP$ elution provided a tailing peak with poor shape (Fig. 4(g)), which led us to conclude that major interactions took place with residual silanols.

It has been reported that the diol behavior is not always predictable when probed with bases due to the presence of the hydroxyl groups of the diol phase, the ether linkage of the packed function and residual silanols [49].

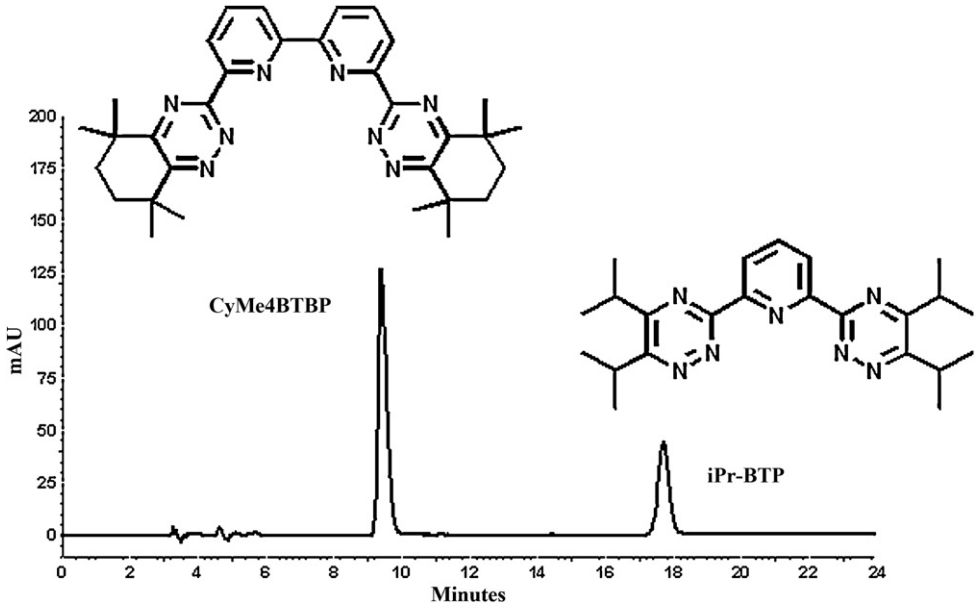


Fig. 6. Separation of $CyMe_4BTBP$ (10^{-4} mol L^{-1}) and iPr-BTP (10^{-4} mol L^{-1}) on the Discovery CN 250 × 4.6 mm column in isocratic elution conditions. Mobile phase: hexane/ethanol 85/15 + 0.1%DEA.

3.1.3. Cyano phases

In our work, very poor chromatographic performances or irreversible $CyMe_4BTBP$ adsorption occurred without basic additives in the mobile phases. Low percentage of diethylamine (DEA, 0.1%) was thus added to the mobile phase in the whole cyano experiments, allowing appreciable improvement of the peak shape. A decrease of the retention time of the solutes was observed, due to the polarity of DEA. When only considering the bonded CN groups as adsorption sites, the dominant retention factors should be dipole–dipole interactions and hydrogen bonds between basic stationary phase and acidic solutes [50,51]. Hence, specific interactions are expected if both the modifiers and the solutes exhibit sufficient dipolarity and/or acidity properties. $CyMe_4BTBP$ exhibits dipolar properties but no significant acidity, therefore predominantly dipolar interactions with the stationary phase might be established. Localization and secondary solvent effects play an increased role with cyano phases, explaining their different behavior regarding diol and amino phases [42,45,49–52]. Hence, depending on the selectivity of the mobile phase, cyano phases can exhibit either acid or basic tendencies, leading to the retention of various types of solutes and making them universal stationary phases. For example, predominantly dipolar compounds such as nitriles and isothiocyanates were retained on cyano stationary phase, even without basic additive [53]. On the other hand, the separation of compounds exhibiting different acid/base and polarity properties has been described [27,30,44,54].

In our work, dioxane was not an adapted modifier, since it led to bad separation or no peak at all. Although polar and localizing solvent, dioxane exhibits basic properties, making it unavailable for sufficient specific interactions with the stationary phase. With other modifiers, it can be observed from Fig. 5(a)–(f) that poor chromatographic performance was obtained with the Luna CN, whereas the Discovery CN led to good results. As in the two previous cases, these results emphasize the different characteristics of two columns provided by diverse manufacturers. With the Discovery CN, we can observe that with 15% of modifier, DMDOHEMA eluted in the injection peak, whereas $CyMe_4BTBP$ was more strongly retained compared to the two previous stationary phases. These data show the higher selectivity of the cyano toward $CyMe_4BTBP$ compared to the amino and diol bonded phases. EtOH and i-PrOH are polar and localizing solvents able to interact with the stationary phase, allowing retention of adsorbing solutes. Using the same percentage of i-PrOH and EtOH, $CyMe_4BTBP$ eluted at 16 and 7.7 min respectively (Fig. 5(b) and (d)). Although basicity, acidity, dipolarity or localization ability of a solvent are important parameters, steric factors seem to play a significant role in separation processes. Indeed, bulkier alcohols (i-PrOH versus EtOH or MeOH) were reported to yield higher retention times regarding the separation of polar basic compounds such as amines with cyano phases [30]. In the same manner, retention times of polar solutes were higher using acetic acid rather than trifluoroethanol in hexane, although these modifiers display similar properties of acidity and polarity [51]. It was stated that acetic acid was a dimmer in hexane and behaved therefore as a weak modifier. A hindered modifier has a reduced accessibility of the stationary phase active sites, inducing weaker interactions with these latter. Therefore, the ability to compete with a solute interacting with the polar sites is decreased, involving higher solute retention time. Narrow peak was obtained with (CH_2Cl_2/MeOH) (R_t = 6.8 min) (Fig. 5(f)). As for the previous cases, CH_2Cl_2 as a non localizing solvent exhibits different properties compared to i-PrOH and EtOH. Different retention processes of solutes might occur, involving secondary solvent effects, which are known for the cyano phases.

In summary, the CN phase exhibited higher selectivity toward $CyMe_4BTBP$ by comparison to the diol and amino phases, highlighting again the difference in the retention mechanisms between cyano and amino/diol bonded phases. Kagan et al. [30] showed that for separation of diverse substituted amines, more complex separation mechanisms took place for the cyano stationary phases than for the diol one. The whole results illustrate again the importance of stationary and mobile phases combination on retention and selectivity in NP-HPLC. They also stress the need to investigate several stationary and mobile phases to separate a same set of solutes.

For further study of $CyMe_4BTB$ hydrolytic degradation by NP-HPLC-MS, Discovery CN column with hexane/EtOH + DEA as mobile phase has been selected. Using this stationary and mobile phases, we managed to separate the $CyMe_4BTBP$ and a polyazaaromatic extracting molecule from the 2,6-bis(5,6-dialkyl-1,2,4-triazin-3-yl)pyridine (BTP) family previously tested in the SANEX process: the 2,6-bis(5,6-di-isopropyl-1,2,4-triazin-3-yl)pyridine (i-PrBTP,

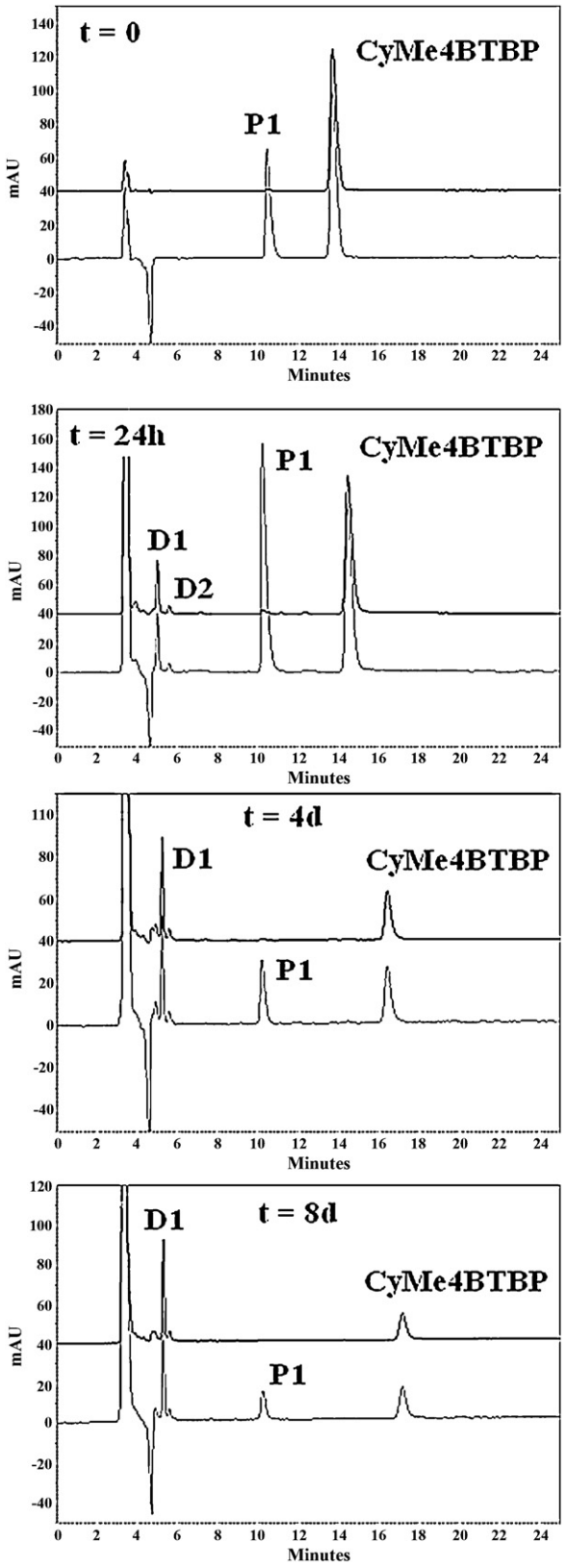


Fig. 7. Monitoring of the hydrolysis of $CyMe_4BTBP$ contained in the organic phase of the octanol/nitric acid 3 mol L^{-1} HNO_3 system with a DAD (230 and 250 nm). Discovery CN 250 × 4.6 mm column, mobile phase hexane/ethanol 85/15 + 0.1%DEA in isocratic elution conditions.

Table 1
Assignment of $CyMe_4BTBP$ product ions obtained from the CID-MS/MS experiments.

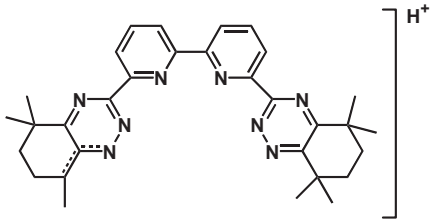
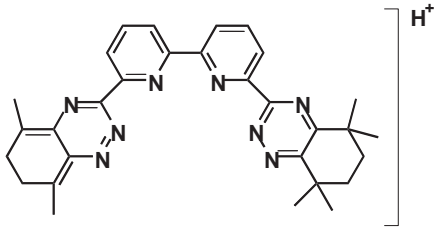
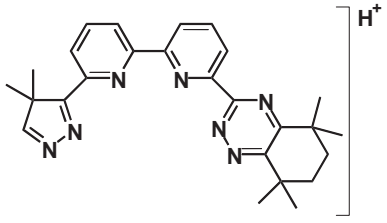
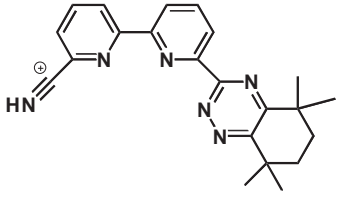
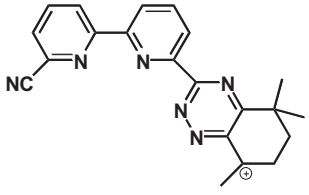
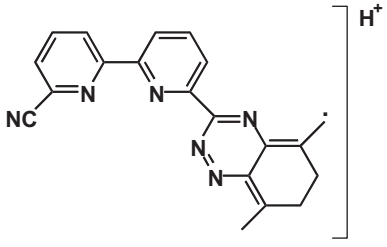
m/z in positive ion mode	Structure
520.2	
505.3	
440.4	
371.2	
355.3	
341.2	

Fig. 6). Although less polar than $CyMe_4BTBP$, i-PrBTP eluted later than $CyMe_4BTBP$. Due to the higher steric hindrance of $CyMe_4BTBP$, it might be suggested that active sites were less accessible for $CyMe_4BTBP$, involving weaker interactions with these latter. Moreover, solute–solvent interaction in the mobile phase should be higher with the $CyMe_4BTBP$, inducing a decreased retention time.

3.2. Hydrolysis of $CyMe_4BTBP$

3.2.1. NP-HPLC-DAD experiments

Experiments were carried out following the procedure described in Section 2. In both hydrolysis conditions (1 and 3 mol L^{-1} HNO_3), a product eluting around 11 min (P_1, Fig. 7) and exhibiting an absorption maximum at 230 nm, was observed at t_0 (contact between the two phases). P_1 displayed stronger relative intensity for 3 mol L^{-1} HNO_3 conditions than for the 1 mol L^{-1} HNO_3. Pure octanol was hydrolyzed under the same conditions, leading to the formation of the same product at t_0, whereas hydrolysis of octanol by HCl did not induce the formation of P_1 (data not shown). Hence, we can deduce that P_1 was the result of the octanol oxidation by nitric acid, as discussed in the following section. In liquid–liquid extraction experiments, it is known that the nitric acid, as oxidative reagent, has an impact on the degradation products yield as well as on their nature, producing additional functionalized compounds of extractants and diluents. The nature of the diluent has also an influence on the degradation process.

During 1 and 3 mol L^{-1} HNO_3 hydrolysis, peaks consistent with hydrolysis degradation products of $CyMe_4BTBP$ emerged. After six days, two minor degradation products were formed for 1 mol L^{-1} HNO_3 conditions, whereas they were observable after 24 h for 3 mol L^{-1} HNO_3, with a major product R_t = 5 min (D_1) and a minor product at R_t = 5.6 min (D_2) (Fig. 7). In stronger acidic conditions, the relative intensity of the peak corresponding to $CyMe_4BTBP$ decreased greatly with time. These observations evidenced the $CyMe_4BTBP$ stability against 1 mol L^{-1} HNO_3, in agreement with previous successive Am(III)/Eu(III) extraction experiments for which the distribution ratios did not show significant change during 1 mol L^{-1} HNO_3 hydrolysis [15].

Due to its basic properties, $CyMe_4BTBP$ is able to extract HNO_3 through several mechanisms. The polarity of the $CyMe_4BTBP$ is therefore increased, involving higher retention time as observed Fig. 7.

3.2.2. Identification of degradation products by NP-HPLC-APCI-MS

The most difficult step in coupling NP-HPLC to mass spectrometry is the optimization of experimental conditions which include the ionisation of analytes in the presence of mobile phase solvents. The use of hexane – an inefficient proton donor which is not ionised under ESI conditions – led to molecules ionisation by APCI-MS. Prior to the coupling experiments, the MS acquisition parameters, under chromatographic conditions (hexane/EtOH, 85/15 + 0.1% DEA) by direct infusion, were optimized for $CyMe_4BTBP$ using this ionisation mode. Due to its very low concentration, the use of DEA in the mobile phase did not involve signal suppression, as it has been already reported in the literature [33]. Under such APCI-MS conditions, $CyMe_4BTBP$ produced protonated molecules $[M+H]^+$ at m/z 535.5, and its DEA adduct ion $[M+H+DEA]^+$ at m/z 608.1; these were confirmed by a series of CID-MS/MS experiments. For example, the $[M+H]^+$ ion at 535.5 led to some characteristic product ions as proposed in Table 1, corresponding to the dealkylation and step-by-step aromatisation of the ion structure.

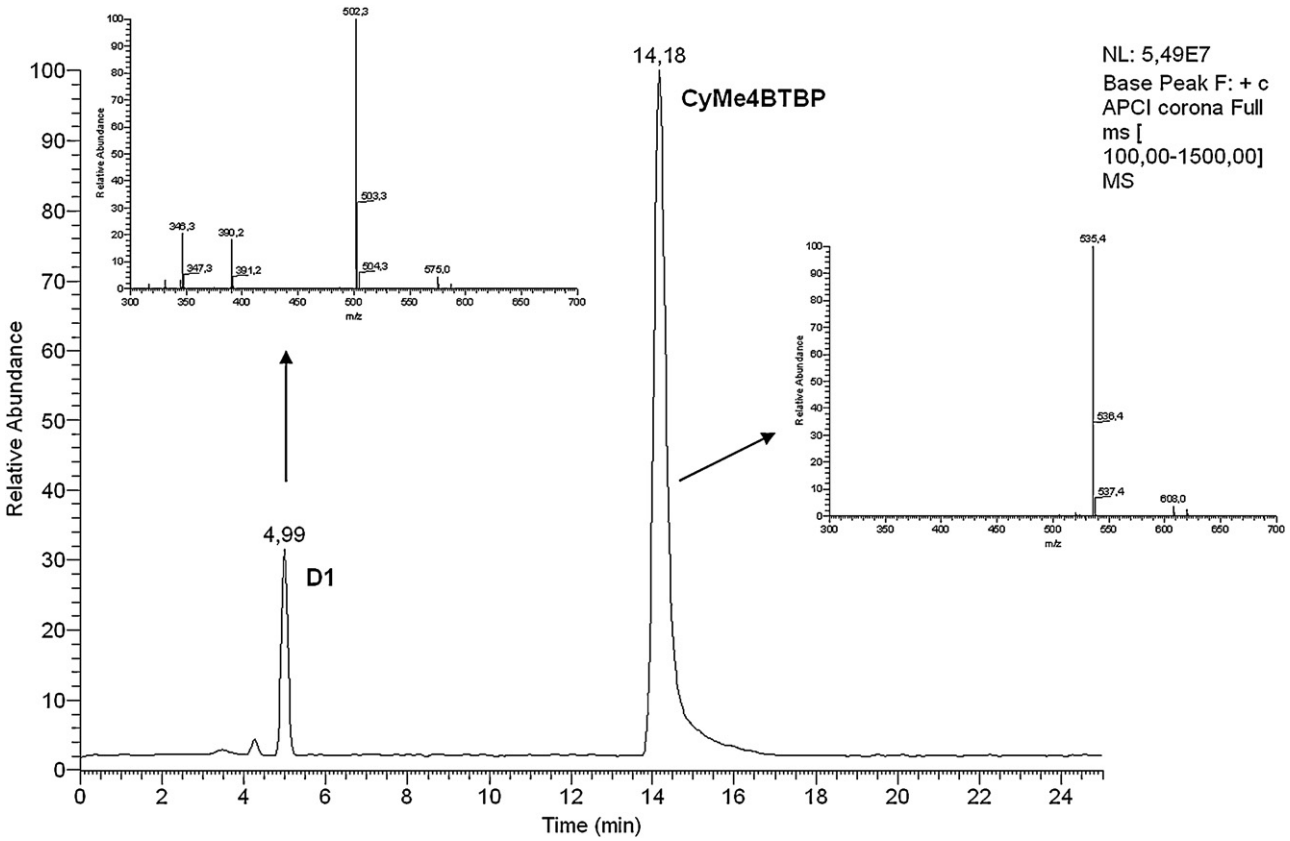


Fig. 8. NP-HPLC-APCI-MS chromatogram (base peak) in positive ion mode of hydrolyzed $CyMe_4BTBP$ (HNO_3 3 mol L^{-1}, t = 36 h) and extracted mass spectrum of each peak ($CyMe_4BTBP$ and the main degradation product D_1). Discovery CN 250 × 4.6 mm column, hexane/ethanol 85/15 + 0.1% DEA in isocratic elution conditions.

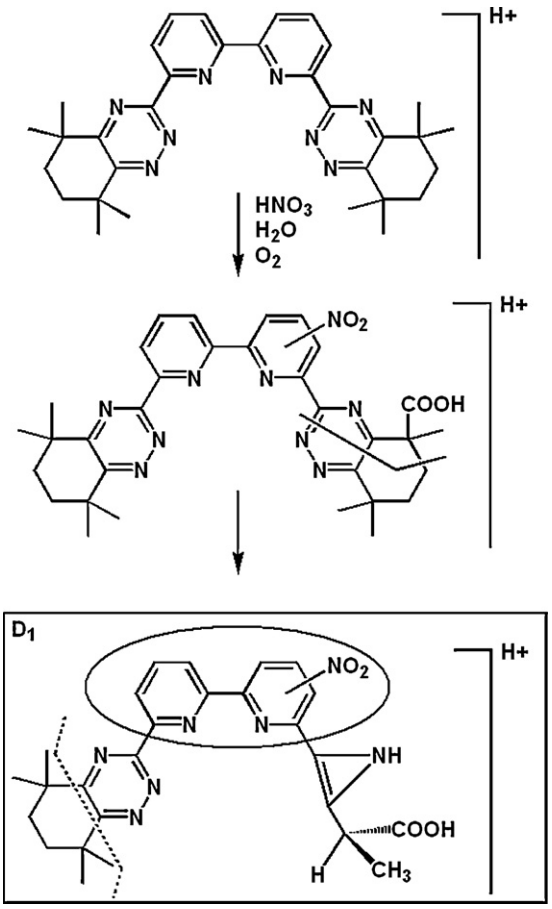


Fig. 9. Possible structure for the main degradation product ion, D_1 identified by NP-HPLC-APCI-CID-MS/MS experiments.

Under 1 mol L^{-1} HNO_3 acid hydrolysis, the degradation pathway of BTP and BTBP bearing linear alkyl chains (such as C5-BTBP) followed the nitration of one CH_2 group as a first step (usually in the α- to triazinyl ring-position) [20]. Further oxidation leads to the corresponding alcohol and ketone derivatives. Additional chemical attack could occur on a second CH_2 group, leading to diols, diones, and/or more oxidized compounds. In addition, alkyl chains could be lost and triazinyl rings could be broken [55,56]. Although the cyclic $CyMe_4BTBP$ exhibits greater acidic hydrolytic stability compared to the C5-BTBP, we have observed in this work a major degradation product of $CyMe_4BTBP$, by NP-HPLC-DAD under 3 mol L^{-1} HNO_3 conditions. Pursuing this study, the NPLC-APCI-MS coupling was used to characterise and identify this main hydrolytic degradation product.

After 36 h of hydrolysis, the main degradation product was observed at 4.99 min, giving the precursor ion at m/z 502.3 (Product D_1, Fig. 8), corresponding to the possible formula $C_{26}H_{28}O_4N_7$. The NPLC-APCI-CID-MS/MS experiments on the precursor ion at m/z 502.3 afforded two major product ions at m/z = 390.1 and 346.2 (also observed in the full scan MS spectrum) and two minor product ions at m/z = 458.3 and 302.3. The CID-MS/MS fragmentation route of the precursor ion at m/z 502.3 and the proposed structures of the formed product ions are reported in Table 2. The observation of even product ions, coming from an odd number of nitrogen products, was quite puzzling and led us to suggest the nitration of $CyMe_4BTBP$ as the first step of the interaction of the compound with nitric acid. Within the framework of studies of calixarene degradation under radiation and/or acidic conditions, nitro derivatives resulting from aromatic substitution of calixarenes have been observed in experiments combining radiolysis and 3 mol L^{-1} HNO_3 hydrolysis [22,24]. It was demonstrated that the nitric acid enhanced the degradation process, directing most of it first towards nitration of the parent calixarene, and then towards further degradation of the aromatic compounds which had been activated by the previous nitration.

Table 2
Assignment of D_1 product ions obtained from NP-HPLC-APCI-CID-MS/MS experiments.

m/z in positive ion mode	Assignment
458.3	$[D_1-CO_2]^+$
390.1	$[D_1-C_8H_{16}]^+$
346.2	$[D_1-(CO_2+C_8H_{16})]^+$
302.3	$[D_1-(C_{10}H_6N_3O_2)]^+$

The product ion at m/z = 458.3 corresponds to the loss of a CO_2 moiety, demonstrating the carboxylation of $CyMe_4BTBP$ through oxidation. The last structure of the product ion at m/z = 302.3 was unusual, and was judged to have been formed as a product ion by loss of a protonated nitrobipyridine moiety. From the subtle play of odd–even masses on the precursor ion at m/z = 502.3 and on these product ions, we were able to determine a possible structure for D_1 as shown in Fig. 9. We were also able to confirm the mechanistic pathway as the one in which the $CyMe_4BTBP$ undergoes mononitration and oxidation of one methyl group into COOH. Then the usual fragmentation takes place, for example *via* the aromatisation, leading immediately to the degradation product at m/z = 502.3. Although the present study confirmed the resistance of $CyMe_4BTBP$ to hydrolysis in concentrated nitric medium, classical degradation mechanisms such as nitration, oxidation and the further cleavage of triazinyl rings have been observed.

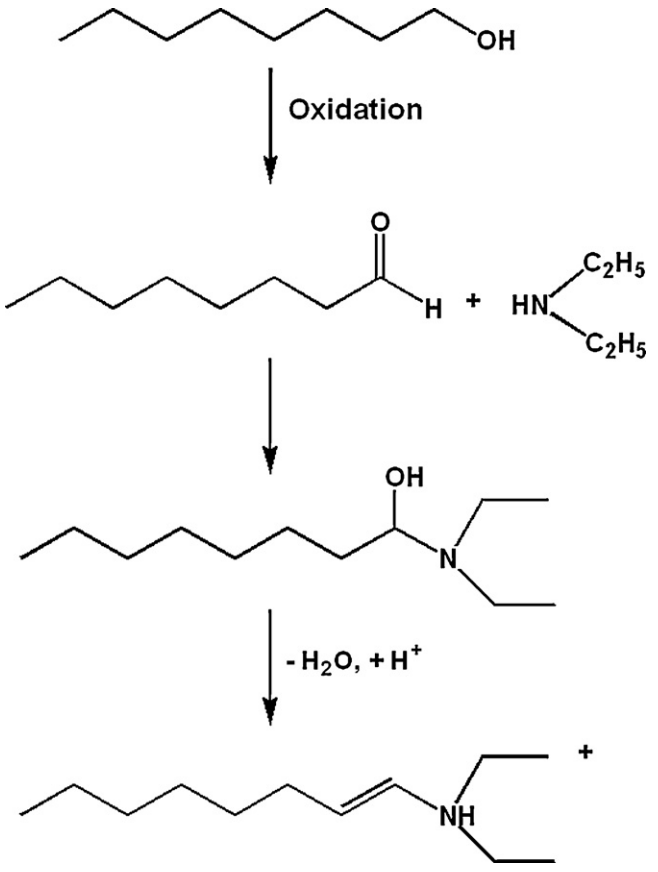


Fig. 10. Structure of the product resulting from the nitric acid oxidation of the octanol in the presence of DEA.

It is also worth noting that the interesting product produced by the nitric acid oxidation of the octanol is observed as a major ion in the LC-MS background to these series of experiments. The major precursor ion at m/z 184 was detected when octanol contacted with nitric acid phase, was eluted on the CN column using Hexane/EtOH, 85/15 + 0.1% DEA. Under such conditions, the oxidation of octanol to the corresponding aldehyde, octanal could take place, followed by the formation of the ene–amine compound (first the DEA addition to the aldehyde, followed by the dehydration of the corresponding amino alcohol to the Schiff base which is isomerised to the final ene–amine adduct compound), giving the even 184 mass (Fig. 10). Incidentally, this fact alone confirmed both the oxidation of octanol under these conditions and the important role played by the DEA through the formation of such a DEA adduct.

4. Conclusion

The aim of this work was to develop a chromatographic method to separate, identify and characterize hydrolytic and radiolytic degradation products of $CyMe_4BTBP$. Within this framework, a simple, fast NP-HPLC-APCI-MS separation method involving polar bonded stationary phases has been set up. This NP chromatographic mode seems to be well suited for the separation of extracting molecules devoted to the nuclear fuel reprocessing and to our knowledge; this is the first time that such an analytical method was developed for this type of study. In all cases, experiments were carried out under isocratic mode of elution, which is a significant advantage regarding analysis time, particularly when coupling with mass spectrometry is performed. The main degradation product coming from hydrolysis of $CyMe_4BTBP$ in octanol by $3\,mol\,L^{-1}$ HNO_3 has been characterized. It has been found that in octanol/nitric phase system, the $CyMe_4BTBP$ degradation led to the formation of a nitro-carboxylic acid compound having only half of the heteroaromatic base 1,2-pyridazine left; the rest of it was rearranged. As far as future applications of this method, more identification and quantification of $CyMe_4BTBP$ radiolytic degradation products will be investigated. Such analytical developments are of prime interest regarding studies of various organic extracting systems used in the reprocessing of nuclear fuel. Since the initial sample treatment is not required, the method presented in this study complied well with this objective.

Acknowledgments

The authors would like to acknowledge the CEA/DEN/DISN/SEPOU program for financial support and C. Hill from CEA Marcoule DRCP/SCPS/LEPS (France) for helpful discussions.

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