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**Tuning retention and selectivity in reversed-phase liquid chromatography by using functionalized multi-walled carbon nanotubes**

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## Tuning retention and selectivity in reversed-phase liquid chromatography by using functionalized multi-walled carbon nanotubes

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### Abstract

Aim of this work is to explore the possibility of retention and selectivity tuning in reversed-phase liquid chromatography by means of chemically modified multi-walled carbon nanotubes (MWCNTs). These were synthesized by derivatizing pristine MWCNTs with amino-terminated alkyl chains containing polar embedded groups. A novel hybrid material based on functionalized MWCNTs (MWCNTs-R-NH<sub>2</sub>) was prepared, characterized and tested. The idea was to dispose of a mixed-mode separation medium basing its sorption properties on the peculiar characteristics of MWCNTs combined with the chemical interactions provided by the functional chains introduced on the nanotube skeleton. MWCNTs-R-NH<sub>2</sub> were easily grafted to silica microspheres by gamma radiation (using a <sup>60</sup>Co source) in the presence of polybutadiene as the linking agent. The composite was characterized by scanning electron microscopy (SEM) and Brunauer, Emmett and Teller (BET) analysis in terms of structural morphology, surface area and porosity. The MWCNTs-R-NH<sub>2</sub> sorbent was tested as stationary phase. The reversed-phase behaviour was first proved by analysis of alkylbenzenes, while the key role of CNT derivatization in addressing the selectivity/affinity towards the solutes was evidenced by testing three classes of analytes, *viz.* barbiturates, steroid hormones and alkaloids. These compounds, with different molecular structure and polarity, were here analyzed for the first time on CNT-based LC stationary phases. The behaviour of the novel sorbent was compared in terms of retention capability and resolution with that observed using unmodified MWCNTs, pointing out the mixed-mode characteristics of the MWCNTs-R-NH<sub>2</sub> material. The same test mixtures were analyzed also on a

conventional mono-modal separation sorbent (C18) to highlight the particular behaviour of the (derivatized)MWCNTs-based stationary phases. The novel material showed better performance in separation of polar compounds, i.e. barbiturates and alkaloids, than the unmodified MWCNTs and than the C18 column. Results showed that MWCNT functionalization is powerful to modulate retention/selectivity in reversed-phase liquid chromatography.

**Keywords:** functionalized carbon nanotubes; liquid chromatography; mixed-mode stationary phases

## 1. Introduction

In the recent years carbon nanotubes (CNTs) have been tested in separation science because of their outstanding properties, not comparable to those of traditional carbon materials (e.g. graphite, graphitized carbon, activated carbon). Indeed, the large surface area, high thermal and mechanical stability, and possibility to exert a number of different chemical interactions (electrostatic forces,  $\pi\pi$ stacking, dispersion forces, dative bonds and the hydrophobic effect) with a large number of solutes make CNTs excellent candidates to design novel chromatographic materials due to the possibility of derivatizing them with both polar or apolar side chains (Herrera-Herrera et al., 2012; Speltini et al., 2013a; Valcárcel et al., 2008). In this context, differently from the common chromatographic reversed-phases that can be regarded as mono-modal separation media, nanostructured carbon materials showed special selectivity (Speltini et al., 2013a). The good sorption properties of CNTs have been exploited to prepare a number of chromatographic stationary phases for both gas chromatography (GC) (Hussain et al., 2011; Merli et al., 2010; Saridara and Mitra, 2005; Speltini et al., 2010, 2012) and liquid chromatography (LC) (André et al., 2012; Chambers et al., 2011; Merli et al., 2015; Speltini et al., 2013b), as recently reviewed (Speltini et al., 2013a; Zhang and Qiu, 2015). The analytical application of these carbon nanomaterials in the field of separation science does not cover the chromatographic technique only, but also includes sample preparation, in particular with regard to solid-phase extraction (SPE) and solid-phase micro-extraction (SPME) (Herrera-Herrera et al., 2012; Socas-Rodríguez et al., 2014).

Chemical derivatization of CNTs proved to be a valid strategy to enhance the sorption capability and selectivity in SPE of metal species (Afzali et al., 2012; Peng et al., 2015; Zhao et al., 2015) and organic

contaminants (Li et al., 2009;; Salam and Burk, 2008; Speltini et al., 2014), but also in GC applications (Merli et al., 2010; Speltini et al., 2010, 2012). In particular, chemical modification of the nanotube skeleton with hydrophobic domains embedding polar moieties was determinant to prepare “mixed-mode” sorbent materials capable of specific interplays with the solutes (Speltini et al., 2012,2014), similarly to the multi-modal phases proposed by Lämmerhofer et al. (2011).

With regard to LC, various approaches have been practiced to support CNTs onto proper silica substrates or to incorporate them into polymeric phases, including both chemical bonding or non-covalent immobilization (Speltini et al., 2013a; Zhang and Qiu, 2015). Recently, a novel method entailing gamma radiation has been proposed to anchor MWCNTs onto silica (Speltini et al., 2013b), and the procedure turned out to be simple, mild, one-pot and maintaining the CNT pristine structure. Being the adsorption affinity of such composite strictly related to the MWCNTs properties, that stationary phase resulted as a double-purpose material, suitable for chromatographic applications, and at the same time a valid tool to study the intermolecular interactions of aromatics with the nanotubes (Merli et al., 2015).

MWCNTs, prepared by inserting polyelectrolytes with quaternary ammonium groups onto the CNT backbone, were electrostatically adsorbed onto the surface of sulfonated polystyrene-divinylbenzene beads to obtain an anion exchanger sorbent for ion chromatography (Huang et al., 2013). To the authors' best knowledge, only one paper is presently available in the literature reporting on reversed-phase LC based whose specific adsorption and selectivity are strictly imparted by the CNT functional groups. Specifically, fluorinated double-walled CNTs were immobilized onto silica to combine the analytical performance of CNTs and the unique selectivity of fluorine based compounds for polar substances (André et al., 2014).

On the basis of the current state of the art, in this paper we report the synthesis, characterization and application of a novel LC stationary phase consisting of silica microspheres derivatized with chemically-modified MWCNTs. These were prepared starting from pristine MWCNTs (purified from the metallic fraction) by derivatization with 2,2'-(ethylenedioxy)diethylamine, and their functionalization degree was determined by thermogravimetric analysis (TGA). Derivatized MWCNTs were immobilized by gamma radiation onto the silica support in presence of polybutadiene (PB) which has the sole function of linking agent (Speltini et al., 2013b). The hybrid material (MWCNT-R-NH<sub>2</sub>-PB-silica) was characterized by scanning electron microscopy (SEM), Brunauer, Emmett and Teller (BET) isotherms and attenuated total

reflectance Fourier transform infrared spectroscopy (ATR FT-IR) measurements, and the batch-to-batch reproducibility was investigated. Barbiturates, steroid hormones and alkaloids, three classes of widely employed drugs of pharmacological interest and with different molecular structure and polarity, were chosen as test analytes to figure out the adsorption properties of the sorbent and to appreciate the role of MWCNT functionalization in comparison with unmodified MWCNTs. The results have been discussed in terms of retention capability, resolution and sorption affinity, and have been compared to those observed on a C18 reversed-phase sorbent, known to be a mono-modal separation phase.

## 2. Experimental Section

### 2.1. Materials and reagents

Pristine MWCNTs (outer diameter 20-50 nm, inner diameter 5-10 nm, length 10-20  $\mu\text{m}$ , specific surface area  $60 \text{ m}^2 \text{ g}^{-1}$ , purity > 95 wt%) were obtained from Cheap Tubes Inc. (Brattleboro, Vermont, USA). Silica microspheres (average particle size 5  $\mu\text{m}$ , average pore diameter 100  $\text{\AA}$ , surface area  $200 \text{ m}^2 \text{ g}^{-1}$ ) were purchased from Agilent Technologies Italia S.p.A (Cernusco sul Naviglio, Italy). Ultrapure water (resistivity  $18.2 \text{ M}\Omega \text{ cm}^{-1}$ ) was produced by a Milli-Q system (Millipore, Milan, Italy). Ultrapure HCl (37%, w/w) was from Merck Chemicals (Milan, Italy).  $\text{H}_3\text{PO}_4$  (85%, w/w) and ethylbenzoate (99%) were from Carlo Erba Reagents (Milan, Italy). Triton X-100, *m*-chloroperbenzoic acid, benzene, toluene, dichloromethane, HPLC grade acetonitrile (ACN), PB ( $M_n$  5000, 80% cis+trans, 20% vinyl), 2,2'-(ethylenedioxy)diethylamine (98%), ethylbenzene (99%) and 2-chlorotoluene (99%) were supplied by Sigma-Aldrich (Milan, Italy). Other chemicals were obtained from Sigma-Aldrich. An Orion 420A pH meter (Thermo Electron, Rodano, Italy) was used.

### 2.2. Preparation of MWCNT-R-NH<sub>2</sub>-PB-silica

Firstly, MWCNTs were chemically modified with amino-terminated alkyl chains and the derivatization was checked by TGA, as earlier reported (Merli et al. 2011; Speltini et al., 2010). MWCNT-R-NH<sub>2</sub>-PB-silica was prepared as described in the following. A modification of a known procedure (Bukanova et al., 1976) was followed to prepare MWCNT-R-NH<sub>2</sub>-PB: 5 g of 1,4-polybutadiene ( $M_n$  5000, 80% cis+trans, 20% vinyl) were dissolved in 100 mL anhydrous benzene. To the solution, 10 g *m*-chloroperbenzoic acid were added

and the mixture was refluxed for 72 h under nitrogen. The suspension was centrifuged to separate the benzoic acid formed; the solution was collected and washed with three aliquots of water (200 mL each). The organic phase was rotavapored to obtain a viscous yellowish liquid. The so obtained epoxide is dissolved in 150 mL toluene and 2 g MWCNTs-R-NH<sub>2</sub> were added. The mixture was refluxed for five days under nitrogen. The suspension was centrifuged, and the precipitate (3.2 g of MWCNT-R-NH<sub>2</sub>-PB, containing the initial MWCNTs-R-NH<sub>2</sub>) was washed four times with 40 mL toluene. The so obtained black mass was dried for 24 h at ambient temperature. To obtain MWCNT-R-NH<sub>2</sub>-PB-silica, 250 mg of MWCNT-R-NH<sub>2</sub>-PB were dispersed by sonication (30 min) in 85 mL dichloromethane, to which 250  $\mu$ L PB and 40  $\mu$ L Triton X-100 were added; after addition of 4.2 g silica, the suspension was magnetically stirred for 30 min, sonicated for 5 min, again stirred for 30 min, and finally the solvent was evaporated under vacuum. The obtained grey powder (MWCNTs-R-NH<sub>2</sub> content 3.6 wt%) was transferred in a glass vial and maintained in high vacuum (10<sup>-5</sup> Torr) for 5 h to remove traces of solvent and oxygen. The vial was glass-sealed while kept under vacuum and then irradiated by a <sup>60</sup>Co source for a total dose of 50 kGy.

### 2.3. Preparation of MWCNT-PB-silica

MWCNTs were purified by HCl treatment as earlier reported (Merli et al. 2011; Speltini et al., 2010); the purified MWCNTs were grafted onto the silica particles by gamma radiation following the procedure previously described (Speltini et al., 2013b), to obtain MWCNT-PB-silica (CNT content 5 wt%).

### 2.4. Characterization of the synthesized materials

ATR FT-IR measurements were performed using a Perkin Elmer 1600 FT-IR equipped with a Specac Mk II Golden Gate system. A total of 256 scans was recorded over 400-4000 cm<sup>-1</sup> (resolution 2 cm<sup>-1</sup>).

Scanning electron microscopy images of the powders were acquired using a Zeiss EVO-MA10-HR microscope. Particle size determination was performed on the raw powders, while morphology was studied on gold sputtered conductive samples. Particle size distribution curves were obtained by the Digital Micrograph (™) 3.11.0 software. Specific surface area measurements on the samples were performed by a Sorptomatic 1990 instrument by Thermo Fisher Scientific. About 0.350 g of powders were charged in the glass sample holder and degassed at 100°C for 60 h. Subsequently, samples were cooled down at -196°C and

2 adsorption–desorption cycles followed by a last adsorption run were performed (B.E.T. method, analyzing gas N<sub>2</sub>, 50 points for run; blank done in He before the 1<sup>st</sup> adsorption run).

### 2.5. Liquid chromatography

The materials were slurry packed into 4.6 ×150 mm HPLC columns, as described elsewhere (Speltini et al., 2013b) and tested as stationary phases. A commercial Hypersil C18 column (4.6 ×150 mm, 5 μm) from Varian (Turin, Italy) was used for comparison. The chromatographic system consisted of a Shimadzu (Milan, Italy) LC-20AT solvent delivery module equipped with a DGU-20A3 degasser and interfaced with a SPD-20A UV detector. Injection volume was 20 μL; elution and detection details are reported in figure captions.

## 3. Results and Discussion

### 3.1. Characterization and properties of the obtained materials

The reaction between epoxidized PB and derivatized MWCNTs has been confirmed by IR spectroscopy. The ATR FT-IR spectrum, recorded on MWCNTs-R-NH<sub>2</sub> linked to the polymer, showed peaks attributable to the N-H bond (2842-2909 cm<sup>-1</sup>) typical of amino and amide groups, a peak at 1683 cm<sup>-1</sup> characteristic of the amidic C=O bond, and two main signals at 1428 and 963 cm<sup>-1</sup> ascribed to PB (see Fig. S1).

The SEM images of the prepared materials (Fig. 1) show MWCNT bundles interlaying the silica particles, which act as supporting material and as spacers. As expected, no evident differences in the morphology were seen in presence of unmodified or functionalized nanotubes. The particle size distributions of the (derivatized)MWCNT-based composites were determined by SEM. Results, reported in Table 1, indicate that the particle size was not significantly different from that of the starting silica (average size 5.4 μm).

From the BET results (Table 1), a decrease of the silica surface area (from ca. 25% to 40%) was observed after derivatization. These variations prove the effective functionalization of silica by MWCNTs through PB linking, with the MWCNT-PB network covering and partially filling the silica particles surface cavities. A six-fold difference in the pore volume between MWCNT-PB-silica and MWCNT-R-NH<sub>2</sub>-PB-silica was also noticed, probably due to the distribution of the MWCNT functional groups in the silica pores.

The characterizations of the novel material showed a good reproducibility in the preparation procedure, as apparent from the results collected on three independent batches of MWCNT-R-NH<sub>2</sub>-PB-silica.



### 3.2. Chromatographic tests

As above anticipated, the aim of this work was to highlight the possibility of selectivity/retention tuning in reversed-phase LC by using chemically modified CNTs. In particular, MWCNTs derivatized with amino-terminating alkyl chains with polar embedded groups were chosen in order to prepare a sort of mixed-mode material able to establish multiple interplays with the solutes, e.g. aspecific interaction, hydrophobic effect,  $\pi$   $\pi$ stacking, dipole-dipole, electrostatic interaction (Speltini et al., 2012).

To begin with, the batch-to-batch reproducibility of the novel material MWCNT-R-NH<sub>2</sub>-PB-silica was assessed by evaluating the capacity factors ( $k'$ ) calculated on three columns packed with 3 batches of the material independently prepared starting from pristine silica (see Section 2.2). Relative standard deviations (RSDs) lower than 15% were obtained (see Table 2). Since the contribution of PB in terms of retention is negligible and the CNT pristine structure is preserved after gamma radiation (Merli et al., 2015; Speltini et al., 2013b), the  $k'$  values experimentally obtained can be ascribed to the intermolecular interplays between solutes and MWCNTs. Good reproducibility in retention times was observed, with RSDs < 2% ( $n=3$ ).

The reversed-phase behaviour of MWCNT-R-NH<sub>2</sub>-PB-silica has been investigated at first by testing a series of alkylbenzenes (Table 3). As it can be seen, the elution order is the same as for MWCNT-PB-silica, with increased retention in going from benzene to *n*-butylbenzene. These findings (mean values of three independent measurements) prove that the material owns reversed-phase characteristics (as expectable). However, remarkably lower  $k'$  values were observed on the mixed-mode sorbent under the same elution conditions (see also Fig. S2). This indicates that MWCNT-R-NH<sub>2</sub>-PB-silica is less hydrophobic than MWCNT-PB-silica, well highlighting the role of CNT chemical functionalization in addressing the retention capability.

In order to better evidence the behaviour of the novel MWCNT-R-NH<sub>2</sub>-based material in comparison with unmodified MWCNTs and to the conventional mono-modal C18 phase, three classes of analytes were tested. Specifically, barbiturates, steroid hormones and alkaloids, compounds of different molecular structure and polarity, were expected to exhibit different affinity for each of the three stationary phases.. All the separations presented in the following have been obtained on each sorbent under the best elution conditions.

Barbiturates are usually separated on C18 columns using mixtures of water and ACN as the eluent phases (Cole 2003), as also described in detail for routine analysis (<http://www.chem.agilent.com/Library/applications/59682635.pdf>). The four drugs tested resulted baseline-separated on the two MWCNTs-based columns, showing symmetric peaks (see Figs. 2a and 3a). The main difference between the two separations is the composition of the mobile phase. Indeed, in the case of MWCNT-R-NH<sub>2</sub>-PB-silica only a little percentage of ACN (<10%) was necessary to achieve complete separation, requiring the same run time. This suggests that, despite the lower interaction with the sorbent, MWCNTs functionalization significantly increased the selectivity towards these amphiphilic molecules consisting of a 2,4,6(1H,3H,5H)-pyrimidinetrione nucleus bearing aliphatic/aromatic substituents. The separation of these drugs on the conventional C18 column (Fig. 4a) was as well satisfactory in terms of resolution and run times, but higher percentage of organic eluent was necessary.

Different chromatographic profiles and elution conditions were observed between the two MWCNTs-containing columns also for the separation of six alkaloids, chosen for bearing to different classes and having a different molecular structure. As apparent from Fig. 2b, the analytes were separated in ca. 30 min on MWCNT-PB-silica but with a marked band broadening and peak tailing for compounds 5 and 6. A gradient elution up to high percentages of ACN (80% v/v) was required to shorten analysis time, thus indicating a stronger interaction between nanotubes and the latest eluting compounds. On the contrary, despite partial overlapping for some analytes, MWCNT-R-NH<sub>2</sub>-PB-silica allowed easy desorption in less than 10 min under isocratic elution (Fig. 3b), using far lower amounts of ACN. Results were better than those obtained on the C18 sorbent. Indeed, on the commercial column compounds 3,4,5 showed large bandwidth and poor resolution, and peak tailing was observed for the two most retained analytes (Fig. 4b) working with ACN-10 mM HCO<sub>3</sub><sup>-</sup> as the eluent (Qiu et al., 2008). This can be ascribed to the different properties of the C18 stationary phase compared to the MWCNT sorbents, as also evidenced by the different elution order observed on the commercial column.

The results so far presented highlight the different adsorption affinity of the two classes of different polarity analytes (barbiturates and alkaloids) for MWCNTs and for C18, and show the advantages of using functionalized MWCNTs.

The influence of chemical derivatization in tuning retention and selectivity is further corroborated by the results obtained in the separation of steroids, compounds having a cyclopentanoperhydrophenanthrene lipophilic core. In particular, various sex hormones (including natural and synthetic androgens, estrogens and progestin) were tested. As it can be seen comparing Figs. 2c and 3c, apart from the different elution order the best chromatographic profile was gained on MWCNT-PB-silica. This can be explained considering that these lipophilic molecules better interact with the unmodified nanotubes with respect the more polar MWCNTs-R-NH<sub>2</sub>. Indeed, the higher affinity of steroids for MWCNTs (indicated by the need of using an organic-rich eluent in the chromatographic elution) accounts for the improved resolution and peak shape.

; Testosterone esters were well resolved on MWCNT-PB-silica (Fig. 2d); on MWCNT-R-NH<sub>2</sub>-PB-silica, these compounds were better separated (Fig. 3d) than the steroids mixture (Fig. 3c). This can be ascribed to the interplays between derivatized CNTs and esters substituent on the steroid nucleus. . As expected for these kind of molecules (Mesmer and Satzger, 1997), all the steroid compounds resulted well separated on the hydrophobic C18 column (Figs. 4c and 4d), although with organic-rich eluents and longer run times compared to MWCNT-PB-silica.

In agreement with previous work about the role of CNT functionalization (Hussain et al., 2009), the derivatization with polar functionality, as in our case, tunes the MWCNTs sorption properties improving selectivity for polar compounds, while the unmodified MWCNTs are better suited for the retention of the apolar and aromatic compounds.

#### 4. Conclusions

A new hybrid material basing its chromatographic properties on derivatized MWCNTs has been prepared, characterized and tested for analytical applications. The synthetic procedure allowed to graft MWCNTs-R-NH<sub>2</sub> onto silica microspheres by gamma radiation, in presence of PB as the linking agent. Characterization data showed the features of the prepared material and proved the chemical immobilization of MWCNT-R-NH<sub>2</sub> onto the micrometric support. The reproducibility of the synthesis has been assessed on three independent batches.

MWCNT-R-NH<sub>2</sub>-PB-silica showed better performance in the separation of highly polar compounds, i.e. barbiturates and alkaloids than the analogue sorbent containing unmodified MWCNTs. This highlighted the

mixed-mode properties of this stationary phase, able to give different and more specific interactions with those solutes. Moreover, shorter run times and lower amounts of organic solvent in the mobile phases were required to obtain acceptable or good resolution. From a chromatographic point of view this novel solid phase performed better than the commercial C18 column for separation of alkaloids. Lipophilic molecules such as steroids were instead better separated on MWCNT-PB-silica, due to their higher affinity for unmodified MWCNTs; as expected for such apolar compounds, the best results were observed on the hydrophobic C18 stationary phase, although with longer analysis times and with higher content of organic solvent. The results indicate that MWCNTs derivatization is a powerful tool for retention and selectivity tuning in reversed-phase liquid chromatography. However, despite the promising results achieved in terms of resolution, selective interaction, run times and chemical stability of the MWCNT-based stationary phases, further work should be addressed to improve column efficiency.

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### Figure captions

**Fig. 1.** Scanning electron microscopy images of (a) MWCNT-PB-silica and of (b) MWCNT-R-NH<sub>2</sub>-PB-silica microspheres: batch 1 (b), batch 2 (c) and batch 3 (d).

**Fig. 2.** Chromatograms obtained on the MWCNT-PB-silica column (4.6 × 150 mm) for a mixture of (a) barbiturates (20-30 mg L<sup>-1</sup>): barbital (1), allobarbital (2) and its impurity (2'), phenobarbital (3), penthotal (4); (b) alkaloids (30-50 mg L<sup>-1</sup>): ephedrine (1), colchicine (2), hyoscyamine (3), vincamine (4), strychnine (5), quinine (6); (c) sex hormones (30 mg L<sup>-1</sup>): methandrostenolone (1), testosterone (2),



medroxyprogesterone (3), progesterone (4), hydroxyprogesterone caproate (5), testosterone propionate (6), stanozolol (7); (d) testosterone esters (30 mg L<sup>-1</sup>): acetate (1), propionate (2), isobutyrate (3), isocaproate (4), enanthate (5) cypionate (6), decanoate (7), undecanoate (8).

Conditions: (a) ACN-water 0.8 mL min<sup>-1</sup>, 18% ACN for 8 min, linear gradient to 27% ACN in 10 min, 214 nm; (b) ACN-25 mM H<sub>3</sub>PO<sub>4</sub> 1 mL min<sup>-1</sup>, 25% ACN for 3 min, linear gradient to 80% ACN in 30 min, 210 nm; (c) ACN-25 mM H<sub>3</sub>PO<sub>4</sub> 1 mL min<sup>-1</sup>, 30% ACN for 3 min, linear gradient to 60% ACN in 30 min, 230 nm; (d) ACN-25 mM H<sub>3</sub>PO<sub>4</sub> 1.2 mL min<sup>-1</sup>, 50% ACN for 9 min, linear gradient to 80% ACN in 30 min, 230 nm.

**Fig. 3.** Chromatograms obtained on the MWCNT-R-NH<sub>2</sub>-PB-silica column (4.6 ×150 mm) for a mixture of (a) barbiturates (20-30 mg L<sup>-1</sup>); (b) alkaloids (30 mg L<sup>-1</sup>); (c) sex hormones (30-70 mg L<sup>-1</sup>); (d) testosterone esters (30 mg L<sup>-1</sup>). For peak identification see Fig. 2 caption.

Conditions: (a) ACN-water (8:92) 1 mL min<sup>-1</sup>, 214 nm; (b) ACN-25 mM H<sub>3</sub>PO<sub>4</sub> (28:72) 0.8 mL min<sup>-1</sup>, 210 nm; (c) ACN-25 mM H<sub>3</sub>PO<sub>4</sub> (37:63) 1.2 mL min<sup>-1</sup>, 230 nm; (d) ACN-25 mM H<sub>3</sub>PO<sub>4</sub> 1.2 mL min<sup>-1</sup>, 45% ACN for 8 min, linear gradient to 60% ACN in 20 min, 230 nm.

**Fig. 4.** Chromatograms obtained on the C18 column (4.6 ×150 mm) for a mixture of (a) barbiturates (20-50 mg L<sup>-1</sup>); (b) alkaloids (30-50 mg L<sup>-1</sup>); (c) sex hormones (30 mg L<sup>-1</sup>); (d) testosterone esters (30 mg L<sup>-1</sup>). For peak identification see Fig. 2 caption.

Conditions: (a) ACN-water (35:65) 1 mL min<sup>-1</sup>, 214 nm; (b) ACN-10 mM NaHCO<sub>3</sub> (90:10) 0.5 mL min<sup>-1</sup>, 210 nm; (c) ACN-25 mM H<sub>3</sub>PO<sub>4</sub> 1 mL min<sup>-1</sup>, 40% ACN for 20 min, linear gradient to 80% ACN in 35 min, 230 nm; (d) ACN-25 mM H<sub>3</sub>PO<sub>4</sub> 1 mL min<sup>-1</sup>, 70% ACN for 10 min, linear gradient to 90% ACN in 30 min, 230 nm.

**Table titles**

**Table 1** Particles size, distribution width, surface area and pore volume of silica microspheres before and after derivatization with MWCNTs or MWCNTs-R-NH<sub>2</sub> (the average particle size of the starting silica declared by the producer is 5  $\mu\text{m}$ ).

**Table 2** Capacity factors ( $k'$ ) for the MWCNT-R-NH<sub>2</sub>-PB-silica batch-to-batch reproducibility.

**Table 3** Comparison of capacity factors ( $k'$ ) obtained on C18, MWCNT-R-PB-silica and on MWCNT-R-NH<sub>2</sub>-PB-silica for alkylbenzenes.

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Sample	Particle size ( $\mu\text{m}$ ) <sup>a</sup>	Distribution width ( $\mu\text{m}$ ) <sup>a</sup>	Surface area ( $\text{m}^2 \text{g}^{-1}$ ) <sup>b</sup>	Pore volume ( $\text{cm}^3 \text{g}^{-1}$ ) <sup>b</sup>
Silica	5.4	0.35	200 <sup>c</sup>	-
MWCNT-PB-silica	5.66	0.16	131	0.43
MWCNT-R-NH <sub>2</sub> -PB-silica (batch 1)	5.62	0.19	137	0.08
MWCNT-R-NH <sub>2</sub> -PB-silica (batch 2)	5.73	0.19	119	0.07
MWCNT-R-NH <sub>2</sub> -PB-silica (batch 3)	5.80	0.15	149	0.08

<sup>a</sup>This work, from SEM analysis.

<sup>b</sup>This work, from BET analysis, uncertainty: 3%.

<sup>c</sup>Producer datum.

Values of capacity factor ( $k'$ ) <sup>a</sup>				
	Batch 1	Batch 2	Batch 3	Mean $k'$ <sup>b</sup>
Ethylbenzoate	0.88	1.12	1.14	1.1(1)
Ethylbenzene	1.39	1.42	1.29	1.4(1)
2-chlorotoluene	2.03	2.77	2.45	2.4(4)

<sup>a</sup> Experimental conditions: ACN-water (40:60), 1 mL min<sup>-1</sup>, 210 nm.

<sup>b</sup> Values in the brackets refer to standard deviation.

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	Values of capacity factor ( $k'$ ) <sup>a</sup>		
	C18	MWCNT-PB-silica	MWCNT-R-NH <sub>2</sub> -PB-silica
Benzene	6.10	1.45	0.66
Toluene	11.02	2.35	1.18
Ethylbenzene	19.20	3.44	1.42
<i>p</i> -xylene	20.31	3.90	2.11
Trimethylbenzene	29.23	6.70	3.60
<i>t</i> -butylbenzene	36.95	7.26	4.04
<i>p</i> -diethylbenzene	49.49	9.56	5.40
<i>n</i> -butylbenzene	54.88	11.01	6.30

<sup>a</sup> Experimental conditions: ACN-water (40:60), 1 mL min<sup>-1</sup>, 210 nm.