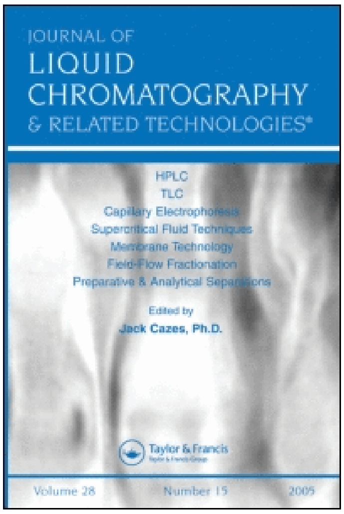

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Flieger, J. , Tatarczak, M. and Szumiło, H.(2006) 'Influence of Zinc and Nickel Sulfates as Mobile Phase Additives on the Retention, Efficiency, and Selectivity of Organic Hydrocarbons with Polar Groups in RP-HPLC', Journal of Liquid Chromatography & Related Technologies, 29: 5, 673 — 683

To link to this Article: DOI: 10.1080/10826070500509223

URL: http://dx.doi.org/10.1080/10826070500509223

Journal of Liquid Chromatography & Related Technologies®, 29: 673–683, 2006

ISSN 1082-6076 print/1520-572X online
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Influence of Zinc and Nickel Sulfates as Mobile Phase Additives on the Retention, Efficiency, and Selectivity of Organic Hydrocarbons with Polar Groups in RP-HPLC

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Abstract: Inorganic eluent additives affect the retention and selectivity of chosen hydrocarbons with polar proton-acceptor groups in reversed phase HPLC. Following that, nickel and zinc sulfates have been used in organic-aqueous mobile phase at the concentration ranging from 0.001 to 0.01 M. The influence of salts is attributed to the desolvation of the analytes mainly due to the sulfate anions in most cases, or selective complexation with particular entities by metal cathions.

The effect of salts concentrations on efficiency on a RP-18-bonded silica column has also been studied.

All investigated compounds displayed significant changes in efficiency with the increase of salt concentration.

Keywords: Antychaotropic mobile phase additives, RP-HPLC, Organic hydrocarbons with polar groups, Retention behavior, Separation selectivity, Efficiency of chromatographic systems

INTRODUCTION

Application of inorganic metal salts in chromatographic research provides many aspects for researchers' consideration. The salts of transition metals have already been applied in classic column chromatography (LC) for improving separation selectivity.

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The salts were highly appreciated alongside the introduction, by Helfferich, of ligand exchange chromatography for separation of small molecules.[1] In this chromatographic technique, the ions of transition metals are immobilized by chelating function groups bound to the matrix.[2]

Initially, the support was ion exchangers and, at present, the microparticulate siliceous or resinous.[3,4]

The key factor in deciding the retention and selectivity of mixture separations is their reaction with the metal present in the stationary phase in the form of a chelate complex. This separation method, adopted for HPLC, is named MIC-Metal Interaction Chromatography. The details of this method and its application were reviewed by El Rassi and Horvath.[5]

Since its very beginning in the 70 s, MIC has been primarily applied for protein separation and purification.[6]

Another option of using selective interactions with metal ions is metal salt addition or addition of their chelate complexes directly to aqueous organic mobile phases.

In the first approach, transient metal salts of Cu(II), Zn(II), Ag(I) were applied in RPLC separation of electron donor substances, such as olefins, heterocyclics, and aromatic compounds.[7,8]

In the second case, anionic forms of solutes, thanks to the creation of an ion pair with an added metal complex, displayed greater retention in the reverse phase system.[9] The application of metal chelates as counter ions appeared more advantageous, in comparison with conventional chromatography of ion pairs. It allowed improvement of selectivity, also in relation to optical isomers.[10,11] Steric selectivity of such complexes as Cu(II)-D or L proline, Cu(II) or Zn(II) aspartame complexes,[12] 4-dodecyldiethylenediamine- Zn(II),[9] was quite high and evidently was connected with the conformationally rigid structures of these chelates.

Another possibility of inorganic salt application in HPLC of small molecules was presented in the series of LoBrutto and Kazakevich.[13–17]

Inorganic chaotropic salts of PF_6^-, ClO_4^-, BF_4^-, $H_2PO_4^-$ were applied as additives to mobile phases in an RP-HPLC system for the analysis of basic analytes. Retention increase was observed, as well as the improvement in peak symmetry of the protonated analytes. Increase of affinity to the stationary phase occurred through the disruption of the solvation shell upon interaction with anions of mobile phase additives. Authors not only stress the importance of an ion pair mechanism, but also the chaotropic effect connected with the changes in structure of water on retention in this method.

The study results point to the disruption in analyte solvation occurring at a lower concentration of counter anions (below 20 mM) and is specific for the type of salt employed.

The authors used a mathematical description of the effect of this solvation-desolvation equilibrium on the HPLC retention factor for calculating solvation parameters of selected protonated basic analytes. This approach has been adapted for the analysis of basic drugs by other authors.[18]

The aim of the present work is to study the influence of zinc and nickel sulfates, used as the mobile phase additive, on retention, efficiency, and selectivity of model hydrocarbons with polar functional groups of differentiated proton donor acceptor character in RP-HPLC separations.

Results obtained were interpreted on the basis of $\log k_1$ vs $\log k_2$ analysis of the chromatographic systems compared.

EXPERIMENTAL

Apparatus

HPLC was performed with a LaChrom (Merck, Hitachi, Germany) chromatograph with a diode array detector. The chromatograms were processed with HP software. The column used was endcapped Purospher Star RP-18, (Merck, Darmstadt, Germany) 55 × 4 mm I.D., particle diameter 3 μm.

Surface areas were calculated by means of the ChemPlus module of the molecular modeling computer program, HyperChem.[19]

Chromatographic Conditions

The retention data were recorded at 20°C using isocratic conditions with a flow rate of 1 mL/min. Peaks were detected at 254 nm. The organic modifier used was methanol. An appropriate amount of salt (zinc sulfate or nickel sulfate) was added to the organic aqueous mobile phase and filtered with a glass microfiltration apparatus equipped with a Cole-Parmer teflon membrane with pore size of 0.5 μm. Final concentration was in the range of 1–8 mM for either nickel or zinc salts.

All analyte solutions were prepared by dissolution in methanol-water to give the concentrations of 0.1–0.2 mg/mL. Injections of 5 μL of these solutions were made. The retention factors calculated were the average of triplicate injections.

Chemicals

The following compounds were used: 1) phenol, 2) m-nitrophenol, 3) o-nitrophenol, 4) 2,4-dinitrophenol, 5). 2,3-dihyroxynaphtalene, 6) 2,5-dimethylophenol, 7) o-cresol, 8) p-nitroaniline, 9) 2-hydroxyquinoline, 10) acetophenone, 11) p-cresol, 12) benzene. Investigated compounds of analytical grade were obtained from P.P.H. Gliwice, Poland.

All solvents, of HPLC grade, were purchased from E. Merck (Darmstadt, Germany).

RESULTS AND DISCUSSION

Nickel and zinc sulfates were used as direct additives to the mobile phase. The chosen salts consist of strong hydrated ions known as "structure makers" or kosmotropes, according to the Hofmeister series.

These ions exhibit a greater change in viscosity with concentration, having positive Jones-Dole's B coefficients. The viscosity Jones-Dole B coefficient equals 0.206 $dm^3 mol^{-1}$ for sulfates.

At the same time, the entropy of hydration for sulfates is negative $-126\,kJ\,mol^{-1}$. Decreases of the entropies of hydration correlate with the tendency for the ion to be excluded in low density water, so that a loss in entropy of the ion in solution is countered by a gain in entropy of water.

The addition of water structuring additives is connected with the increase of surface tension of water organic eluent from 30.57 $N\,m^{-1}$ for 45% MeOH/H_2O to 35.67 $N\,m^{-1}$ for the enriched phase of 0.01 M of sulfate concentration.

Creation of cavities by the analytes in an ordered structure of the solvent depends on the free energy of the system described by the following formula:

$$\Delta G_C = (\gamma^\circ + \delta m) \times A'$$

where δ is surface tension, A' surface area, γ° surface tension in pure water, and m is the salt molality.

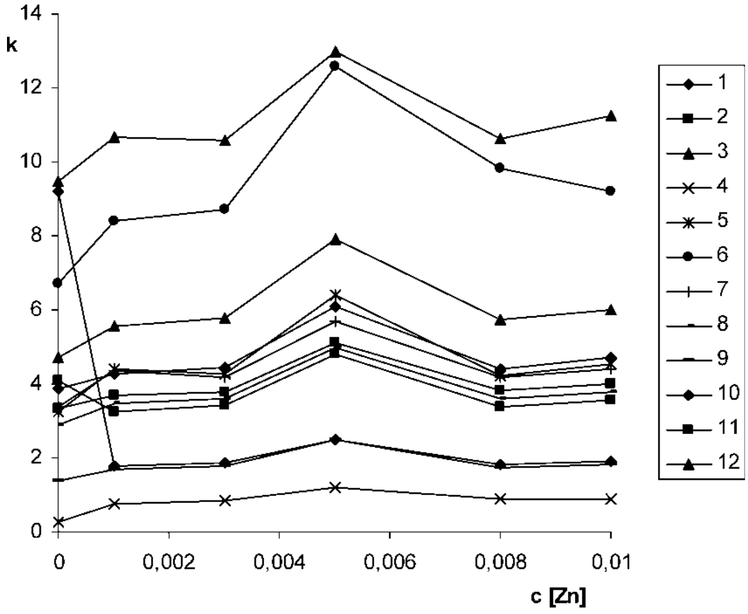

Figure 1. Correlation between retention factor and concentration of zinc sulfate in 45% methanol-water as mobile phase.

Kosmotropic salts increase the value of ΔG_C through their increased surface tensions. The greater A′ value of the investigated analytes, the stronger will be the elimination from the mobile phase and greater retention.

The use of strongly antichaotropic salts as the additives of the mobile phase in the analysis of slightly ionized analytes offers the possibilities of the evaluation on how changes in the structures of water-organic mobile phases influence chromatographic parameters.

The effect of zinc(II)- and nickel(II)- sulfate concentration in methanol-water mobile phase on retention is presented in Figures 1 and 2, respectively.

Up to 5×10^{-3} M of zinc salt, the retention generally increases, followed by a decrease which is observed particularly for the most strongly retained compounds.

In the case of nickel, we may notice a characteristic sharp increase in retention factor in the low concentration region.

Further increase of salt concentration does not affect retention. In both cases, only phenol and m-nitrophenol behave differently. The application of salt modified mobile phases causes a violent decrease of phenol retention, because of the creation of well soluble phenolanes of lower affinity for the stationary phase.

In the case of m-nitrophenol, the lowering of retention was observed only in the phase with the addition of $ZnSO_4$. Similarly, as in the case of

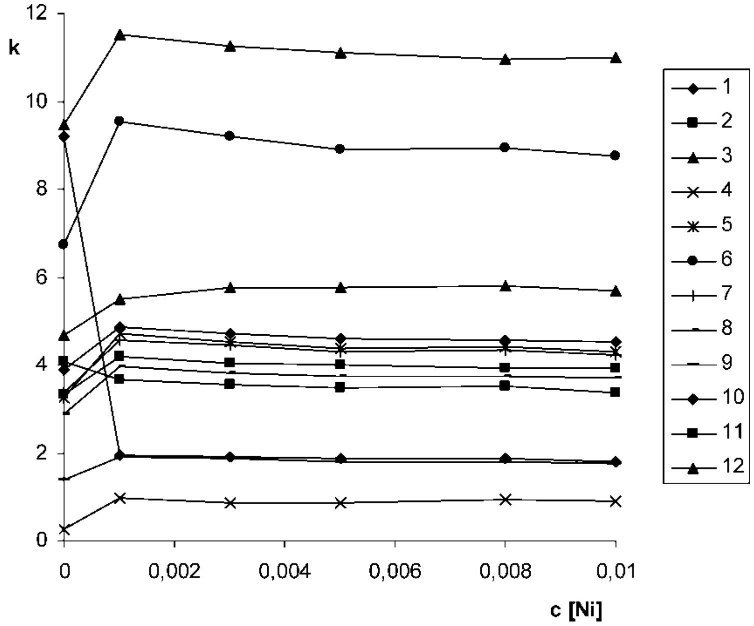

Figure 2. Correlation between retention factor and concentration of nickel sulfate in 45% methanol-water as mobile phase.

phenol, such behavior is determined by the nature of the cation. Zinc, owing to its coordination flexibility, creates two-ligand complexes with m-nitrophenol. The high coordination valency of zinc is supplemented with water molecules, which increases their hydrophilicity and causes the decrease of retention.

By contrast, nickel, presenting lower coordination valency, creates square complexes of lower hydrophilicity.

Both, in the case of $ZnSO_4$ and $NiSO_4$, the greatest increase of retention connected with the addition of salt is observed for the compounds of greatest surface area that is proportionate for 2,5-dimethylphenol (289.9), 2,3-dihydroxynaphtalene (255.12), and for p-cresol (260.94).

Retention increases for these compounds, i.e., $\Delta k = 6.12$, 3.17, and 1.76, respectively, in the phase with the added 0.001 M $NiSO_4$. Comparison of selectivity of modified and unmodified mobile phases is presented in correlation plots, log $k_{mod.}$ vs log $k_{unmod.}$ in Figures 3 and 4.

The slopes of correlation lines (less than 1) in the direction of phases modified with salt testifies to an increase in affinity to the stationary phase in these chromatographic systems. After phenol exclusion and m-nitrophenol, correlation dependencies are described by the following equation: first for correlation of log $k_{45\%\ MeOH}$ vs log $k_{45\%\ MeOH/0,001\ M\ NiSO4}$

$$y = 0.7962 \times -0.2092, R^2 = 0.9965$$

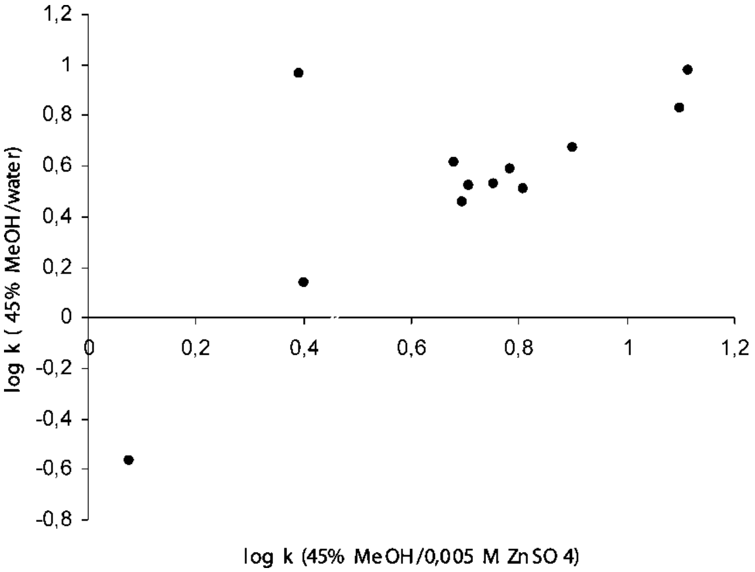

Figure 3. Correlation Log k_1 vs Log k_2 for two eluent systems: unmodified (45% methanol-water) and modified by 0,005 M $ZnSO_4$, compared.

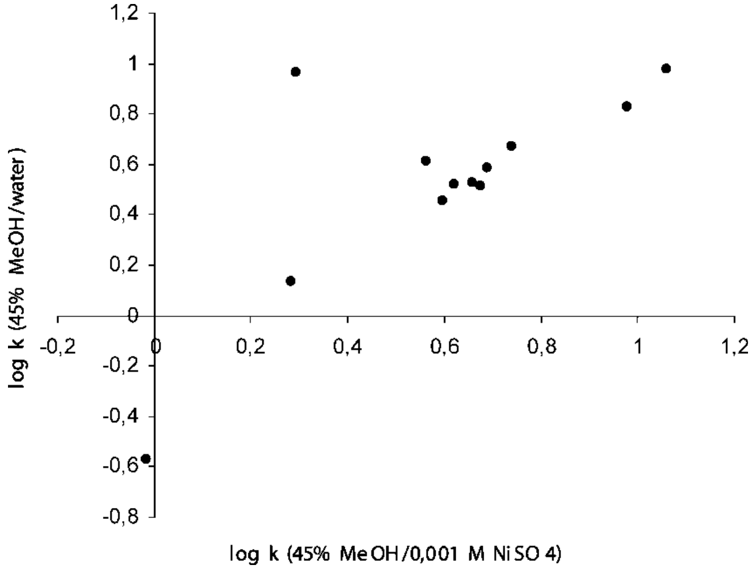

Figure 4. Correlation Log k_1 vs Log k_2 for two eluent systems: unmodified (45% methanol-water) and modified by 0,001 M $NiSO_4$, compared.

second, for correlation of log k $_{45\%\ \mathrm{MeOH}}$ vs log k $_{45\%\ \mathrm{MeOH}/0{,}005\,\mathrm{M}\ \mathrm{ZnSO4}}$

$$y = 0.7267 \times -0.8016, R^2 = 0.9876$$

The increase of surface tension of the water-organic solvent, owing to water structuring additives, makes such a phase more sensitive to minimal differences in hydrophobicities of the analyzed compounds. Thus, the separation factor for m-nitrophenol and o-nitrophenol rises from $\alpha = 1.14$ for water-organic mobile phase, to $\alpha = 1.65$ for the phase with the addition of $NiSO_4$, and $\alpha = 1.66$ for the one with the addition of $ZnSO_4$. For the consecutive structural isomer pair of o-cresol and p-cresol, the α coefficient increases from 1.00 for water-organic mobile phase, to 1.12 for the phase with the addition of $NiSO_4$, and 1.10 for the one with the addition of $ZnSO_4$, respectively.

The minimal difference in hydrophobicity results, in this case, from the influence of the methyl group on the phenol acidity. In the para position, we deal with a positive inductive effect and, in the ortho position, additionally, we deal with the Baker-Nathan effect, known as hyperconjugation. The experimental log P[20] for the ortho isomer is 1.95 and for the para isomer it is 1.94. So, the influence of the antichaotropic additives upon the mobile phase is mainly connected with changes of the aqueous phase and its structure and lipophilicity. We can observe the following rule: the bigger the difference in the hydrophobicity, the bigger will be the retention factor caused by stronger elimination from the mobile phase. For benzene, with logP = 2.13, the difference between retention factors obtained in unmodified mobile phases, and those modified by zinc and nickel sulfates are 1.66 and 3.51, respectively. For toluene, with logP = 2.73, which is very close to the previous one, these coefficients possess quite higher values, i.e., 8.82 and 5.91.

The greatest differences in separation coefficients were observed for benzene and phenol, whose α values in the non-modified system are 1.03, and in the modified system, increases to 5.9 and to 5.25 for mobile phases with the addition of $ZnSO_4$ and $NiSO_4$ respectively.

Such an increase in selectivity is conditioned, not only by lowering of retention as a result of creation of soluble phenolanes, but also retention increase of benzene caused by the addition of antichaotropic salts.

The changes in efficiencies of the studied chromatographic systems are expressed by the plate number, N, in relation to concentration of salt presented in Figures 5 and 6.

The efficiency improvement is observed for the lower solutions of salt, i.e., 0,001 M $NiSO_4$ and 0,005 m $ZnSO_4$. In the last diagram, Figure 7, the comparison of the efficiency obtained in the water-methanol eluent without the addition of salt, and with the addition of salt, with the phase acidified with the acetic acid routinely applied for efficiency improvement minimally dissociating proton-donor compounds with the aim of ion suppression.

As results from the presented measurements, the addition of antichaotropic salt causes even a two-fold increase of the plate number.

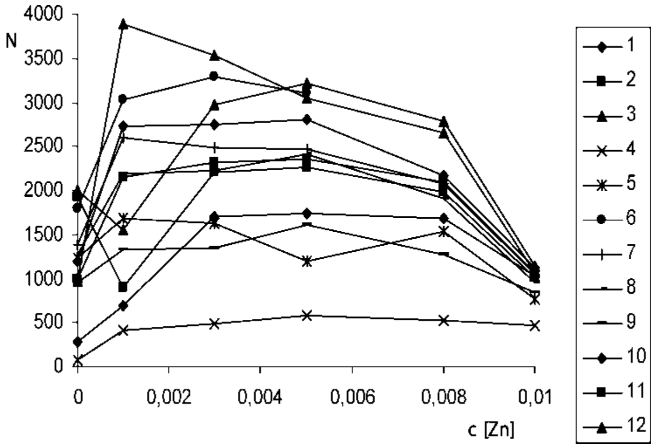

Figure 5. Relationship between plate number (N) and molar concentration of zinc sulfate in 45% methanol-water as mobile phase.

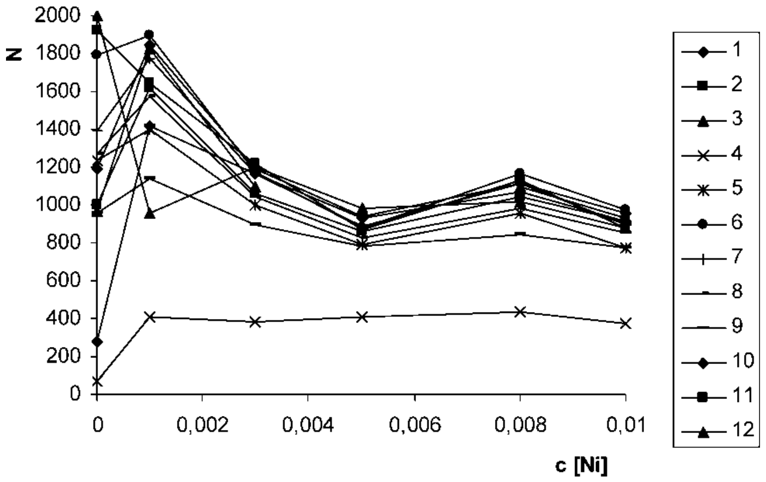

Figure 6. Relationship between plate number (N) and molar concentration of nickel sulfate in 45% methanol-water as mobile phase.

Salt addition, through its negative entropy effect, orders the water structure. From the point of view of the chromatographic system's efficiency, this appears to be much more advantageous than regulation of ionization degree by acid addition.

As can be seen, the rule which is applied in the analysis of protein compounds may be quite useful in chromatography of small molecules, not only from the point of view of efficiency improvement, but also the selectivity of separations obtained.

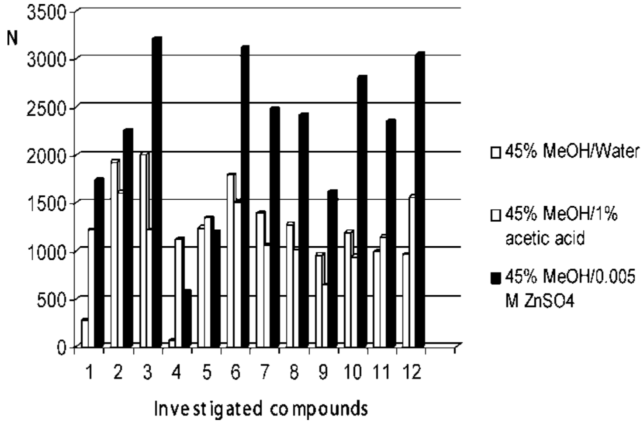

Figure 7. Comparison of N values obtained in different eluent systems for investigated compounds.

CONCLUSION

The application of salts consisting of strongly hydrated ions as additives to the mobile phase in RP-HPLC, in the analysis of small molecules, may have many practical applications.

Firstly, salt addition in very minimal concentrations causes a clear increase in efficiency of the chromatographic system. At such low concentrations, there is no dire need for buffering of the mobile phase to prevent cation hydrolysis. This does not complicate the system and enables more direct interpretation.

Antichaotropic salt orders the structure of the eluent, increases its surface tension, which in turn increases the retention proportionately to the hydrophobicity and surface area of the investigated analytes.

The dependence of retention in the function of salt concentration may have a different course. For the created complexes or soluble salts, we observe a violent lowering of retention resulting from the increase of hydrophilicity. In other cases, entropy changes act as the main driving force and cause chromatographic retention increases in the range of lower concentrations of the added salt.

The strong advantage of the application of transition metal salts is the possibility of selection of the compounds creating complexes in conditions of the chromatographic system applied.

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Received May 3, 2005
Accepted June 12, 2005
Manuscript 6653