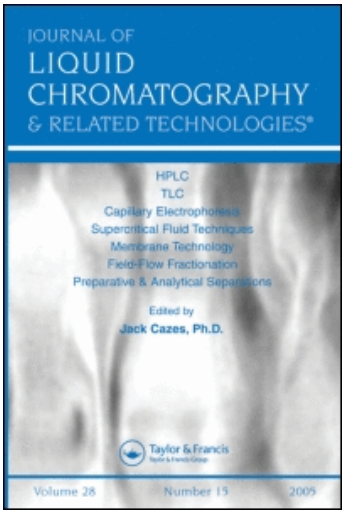

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M. C. Gennaro[a]
[a] Dipartimento di Chimica Analitica, Università di Torino, Torino, Italy

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GHOST, VACANCY, DIP, OR SYSTEM PEAKS? A CONTRIBUTION IN INVESTIGATIONS ABOUT "INJECTION" AND "SYSTEM" PEAKS IN LIQUID CHROMATOGRAPHY

M. C. Gennaro
Dipartimento di Chimica Analitica
Università di Torino
Via P. Giuria
5 - 10125 Torino, Italy

ABSTRACT

Chromatograms obtained by IIR (ion interaction reagent) HPLC technique are generally characterized by the presence of up to two peaks apparently spurious,which are additional with respect to the number of the components of the injected sample. Different explanations were presented by various authors about their formation.

In this paper an interpretation is proposed and supported by experimental results, obtained by employing different ion interaction reagents in different chromatographic conditions.

The formation of the system peaks, namely injection peak and system peak,is explained through the taking place of the same equilibria which determine the retention mechanism of the analyte through this technique.

INTRODUCTION

Reverse phase ionic chromatography RP IC HPLC has recently found much interest,mainly because of its high flexibility and versatility. Different experimental conditions can in fact be properly changed in order to find out the most suitable ones for that particular separation problem to be solved. It becomes so possible to get out the identification and separation of many anions, both organic and inorganic.

It can be shown out that the most determining condition is the proper choose of the eluent flowing through silica base reverse phase column.

Nevertheless, a trouble which is generally present in practically all the systems studied by means of this technique is the presence in chromatograms of additional peaks by respect to the number of components of the injected sample.

Many Authors(1-14) observed in their chromatograms peaks of this kind and they named them with suggestive denominations as "vacancy peaks ", "dip peaks","pseudo peaks" or "ghost peaks".

Even if this last denomination at first sight should seem to be the most suitable for these peaks, if one takes into account their capability to appear and disappear, to change size and sign, the denomination "system peaks" is the preferable one.In

fact they strictly depend on the overall of the conditions of the utilized system, namely on the column and the chemical properties, concentration and pH of the eluent.System peaks do depend on the solutes too or ,better, on the modifications that the injection of solute can induce into the equilibrium established between the column and the eluent.Recent papers (12-14) report probatory and supported interpretation to their formation;nevertheless many points are still to be cleared off.

We too in our chromathographic studies met peak systems and we devoted some studies and experiments to their interpretation.These were carried out using different eluents, namely tetrabutylammonium (TBA) phosphate,TBA chloride,octylamine (OCT) chloride,OCT phosphate,OCT nitrate,OCT salicylate at different concentrations and flows (table I) .No additional component, such as buffer solutions or other,was used;in these conditions the interpretation of the mechanism seems simplified, by respect to other studies in which more complex eluent systems are used (13,14); apolar silica base octadecyl columns were the stationary phase. Both spectrophotometric and conductometric detectors were employed :the comparison of the "system peaks" obtained in these different detection conditions is in many instances conclusively advantageous for their interpretation.

TABLE I

Typical retention time of injection peak and system peak for some ion interaction reagents(IIR).For comparison, retention times for nitrate ions are also listed .

IIR	j.p.	s.p.	NO_3^-
TBA phosphate 0.0200 M; flow 2.0 ml/min; detection : UV (205 nm)	4.0 ±0.5	6.0 ±0.6	32.4 ± 0.9
TBA chloride 0.0200 M; flow 2.0 ml/min; detection : UV (205 nm)	4.0 ±0.5	12.5 ±0.8	20.6 ± 0.4
TBA chloride 0.0100 M; flow 2.0 ml/min; detection UV (205 nm)	4.0 ±0.5	9.5 ±0.5	13.5 ± 0.5
OCT chloride 0.0050 M; flow 2.0 ml/min; detection:conductometric and UV (205 nm)	1.0 ±0.3	7.0± 0.8	12.5 ± 0.3
OCT phosphate 0.0010 M; flow 2.0 ml/min; detection : UV (205 nm) 0.1	2.2 ±0.5	7.0 ± 0.8	8.1 ±0.1
OCT phosphate 0.0010 M flow 2.0 ml/min detection:conductometric	2.2± 0.5	-	
OCT nitrate 0.0050 M flow 2.0 ml/min detection:conductometric	1.0 ±0.4	9.2 ± 0.7	-
OCT salicylate 0.0050 M flow 2.0 ml/min detection :conductometric column C-18 10 µm	1.0± 0.5	16.5 ± 0.5	
OCT salicylate 0.0050 M flow 2.0 ml/min detection :UV (254 nm) column C-18 10µm	-	16.5 ± 0.5	
OCT salicylate 0.0050 M flow 2.0 ml/min detection: conductometric column C-18 5µm,spherical	1.0 ± 0.5	20.5 ± 0.9	

OCT = octylamine ; TBA = tetrabutylammonium

EXPERIMENTAL SECTION

Apparatus

Analysis were carried out by a Varian LC 5060 Chromatograph,equipped with a Vista 401 Data system and a UV-100 spectrophotometric detector.Alternatively a conductometric Wescan 213 A detector was employed: 1 V exit was used,in order to interface it to Vista 401 Data System.

Different commercial HPLC columns were used,namely Merck Hibar RP 250 - 4 RP -18 (10 m), Waters Assoc.Bondapak RP-18 (10 μm) and Brownlee Labs ODS - 224 - Spheri- RP 18 (5 μm,220 x 4.6 mm).

A Waters Assoc.guard column was used throughout with Waters Assoc. Bondapak C18 Corasil (37-50 μm) as packing material.

An Orion 811 pH-meter equipped with a combined glass-calomel electrode was employed for the pH measurements.

A spectrophotometer Hitachi 150-20 was used for the determination of molar absorptivity of salicylate at 254 nm.

Chemicals

UP water (Millipore Milli-Q) was used for the preparation of solutions.Pentylamine,exylamine,octylamine and tetrabutylammonium hydroxide were "Fluka" analytical grade

reagents.Salicylic acid and all the other reagents were "C.Erba" analytical grade chemicals.

Solutions to be used as eluents were prepared by dissolving in UP water the weighed amount of each amine and bringing the solution to a pH value of 6.2 ± 0.3 by adding respectively the acid containing the anion of the salt to be prepared.In order to condition properly the system ,eluent was flowed through the column until a stable baseline was obtained.Generally, time not less than about an hour was necessary.Eluents solutions were fresh prepared each second day.

RESULTS AND DISCUSSION

The discussion about the nature of the system peaks is obviously strictly connected with the mechanisms which give rise to the retainment of the solute on the column. At this light it can be shown that system peaks, as above mentioned,do depend on the modifications that the injection of solute induces into the equilibrium established between the column and the eluent. Our data make agree with the model of the double layer ionic adsorption associated with a step in which electrostatic forces act.According to this model,the ion interaction reagent is

physically adsorbed on the adsorbent surface of the apolar stationary phase.Namely the hydrophobic cation C^+ is adsorbed in the inner Helmholtz plane(IHP) ,in terms of Stern-Gouy-Chapman theory (15) and the position of closest approach between the ions C^+ and their counter anion A^- is in the outer Helmholtz plane.This situation leads to postulate for the ion interaction reagent CA a form,indicated with CA(ads) which is adsorbed on the apolar stationary phase and at the same time partecipates to a dynamic equilibrium with its own ions, contained in the flowing eluent (13,16):

$$CA(ads) \text{ ====== } C^+ + A^- \qquad (1)$$

When eluent fluxes through the reverse phase column and the equilibrium is gained, the eluent adsorbed on the column can hardly be distinguished from the original stationary phase : that is to say that the form CA(ads) can be considered as a dynamic coating of the column. When equilibrium (1) is reached and a solute is injected, the equilibrium (1) can or not be affected.

According our hypothesis,the mechanisms which determinate the formation of the two mentioned "spurious" peaks depend mainly on the capability of solutes to partecipate to equilibrium (1).The species that do not partecipate, will be unretained and coming out as void volume will give rise to the so called "injection

peak".Width and sign of this peak depend on the additive result of the relative contributions to the detected property (absorbance or conductance) of the unretained species.It must be pointed out that"void" volume in this chromatographic technique does not depend only on the column but on the whole chromatographic system(formed by both column and IIR).

On the contrary, the possibility for a solute $\bar{S}$ to be retained is ruled out by its possibility to partecipate to equilibrium (1), i.e. to compete with $\bar{A}$ for $\overset{+}{C}$.The chromatographic retention of the sample $\bar{S}$ proceeds through a preliminar step of electrostatic attraction and a following sorption of the lipophilic portion of the sample molecule onto the apolar surface; so that a CS(ads) form is adsorbed onto the stationary phase, with the same type of mechanism through which CA(ads) is adsorbed.The result of the two competing equilibria :

$$C^+ + A^- \text{ ====== } CA(ads) \qquad (1)$$

$$C^+ + S^- \text{ ====== } CS(ads) \qquad (2)$$

is that, whilst a certain amount of CS(ads) is adsorbed on the apolar phase, a corresponding amount of CA(ads) is desorbed.So that, when the retention time proper of CA(ads) is gained, a negative peak will appear due to the decreased concentration of CA(ads), with respect to the eluents conditions in which baseline was recorded.

As it concerns retainment time, sign and size of the peak corresponding to the S species, these parameters will of course depend on relative properties of the adsorbed form with respect to the IIR and its dynamic equilibrium determining the eluent background,as well as the property which is detected.

On the contrary, the peak system will be always at the same time for the same experimental conditions ,independently from the anion or the anions S^- of the sample: it is generally peak is of negative sign, taking into account that CA(ads) concentration always decreases when a CS(ads) species forms. As it will later shown, the only case which has been envisaged for a system peak of positive sign is when eluent ions themselves at concentration higher than in the eluent are injected.

Some experiments are now presented in order to support the validity of the proposed retention mechanism.

Figures 1,2 and 3 show the effect of injection of ultrapure water(100 microliters) in some systems,namely OCT chloride 0.0050 M,conductometric detection (figure 1) ,TBA chloride 0.0200 M,UV detection (λ = 205 nm) (figure 2) and OCT salicylate 0.0050 M,UV detection (λ = 254 nm),column C-18 10 µm (figure 3 a),conductometric detection,column C-18 10 µm (figure 3 b) and conductometric detection column C-18 5 µm spherical (figure 3 c).

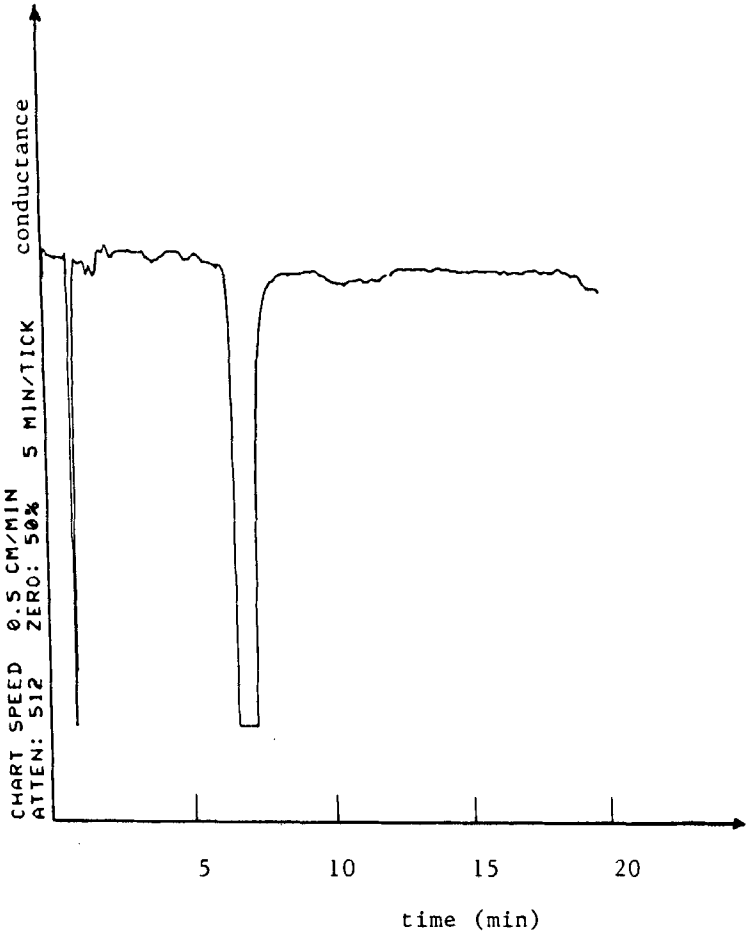

Figure 1 -
Conditions:Column RP C-18 , 10 µ m;IIR = octylamine (OCT)-chloride 0.0050 M; flow 2.0 ml/min; detection : conductometric.
Sample : 100 µ l of UP water.

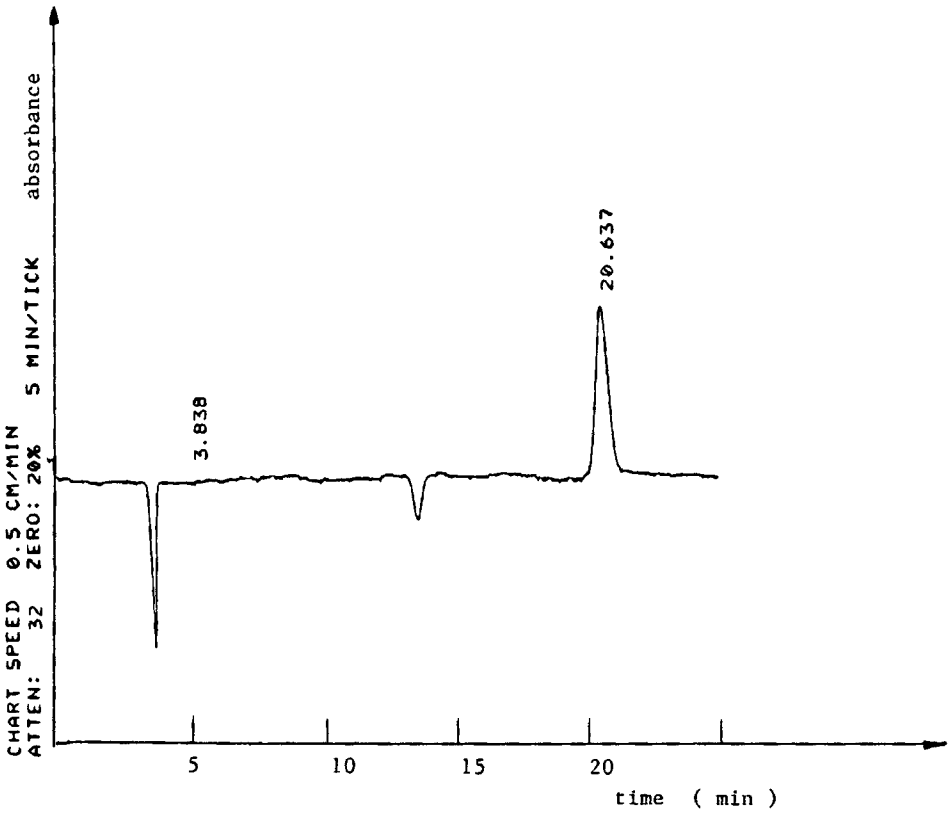

Figure 2 -
Conditions:Column RP C-18 , 10 µ m;IIR = tetrabutylammonium (TBA)-chloride 0.02000 M; flow 2.0 ml/min ; detection : UV (205 nm).
Sample : 100 µl of UP water.

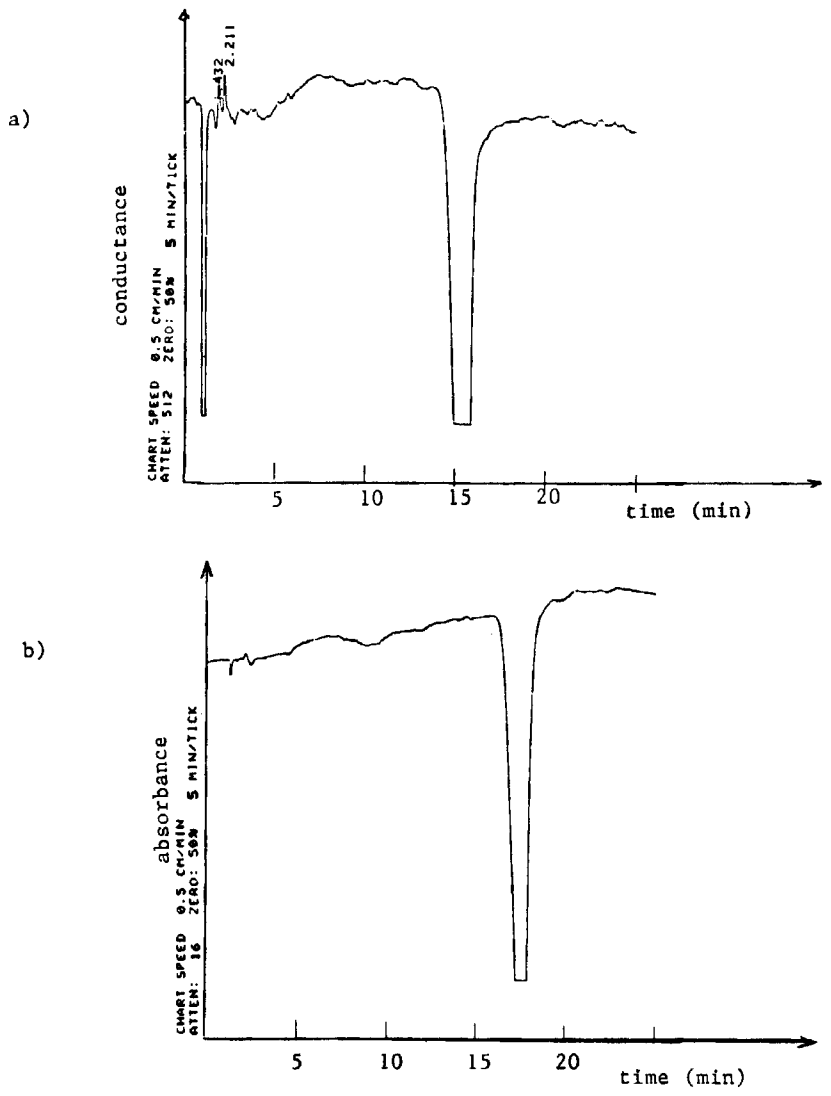

Figure 3 -
Conditions:IIR = octylamine(OCT)-salicylate 0.0050 M; flow 2.0 ml/min
a) column C-18 10 µm;detection : conductometric.
b) column C-18 10 µm;detection :UV (λ= 254 nm)
c) column C-18 5 µm spherical;detection: conductometric
Sample : 100 µ l of UP water

c)

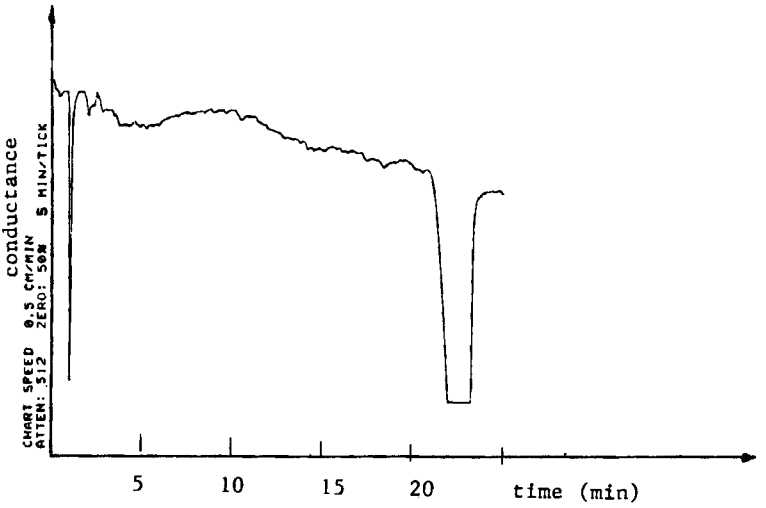

Figure 3 (continued)

In any case water injection gives rise to dilution of the C^+ and $\bar{A}$ ions in equilibrium with CA(ads) with a consequent lowering of property (conductance or absorbance) with respect to the recorded baseline. So that if the sample injected is only pure water a negative injection peak has always to be expected. So for example when OCT -salicylate is used as eluent, due to its high molar absorptivity ($\varepsilon = 308 \pm 2$ $mol^{-1}.cm^{-1}$), water injection produces a more evident variation when a conductometric detector (figure 3 b) is used rather than an U.V. one at 254 nm (figure 3 a). The size will depend on the specific variation introduced by dilution in the detected property. Furthermore, water injection

gives rise,besides the injection peak, to a system peak too,in consideration that dilution acts in shifting equilibrium (1) towards a partial desorption of the CA(ads) species.

As expected,times of the system peaks do depend on the characteristics of the used system.

It can be of interest to observe the chromatogram of figure 4,obtained after injection of 30 µliters of nitrates (10 ppm) into the system OCT-chloride 0.0200 M(conditions as in figure 2) Besides the already discussed injection and system peaks,a positive(increase of conductance) peak due to nitrates is present.

Alkaline or alkaline earth cations do not affect the eq.(1) and, unretained, contribute to the formation of the injection peak .Of course, their contribution will be observable only when conductometric detector is employed.

If the ions not retained contribute to the monitored property at different extent , the resulting injection peak will reflect this situation.So for example,in a system in which the IIR is OCT-salicylate (0.0050 M) and conductance is the detected property,solutions (100 microliters) containing increasing amounts of NaCl were injected and chromatograms compared with that obtained for injection of water only.When NaCl concentration is 10.0 ppm (figure 5),the increase of conductance due to the added electrolite practically compensates the effect

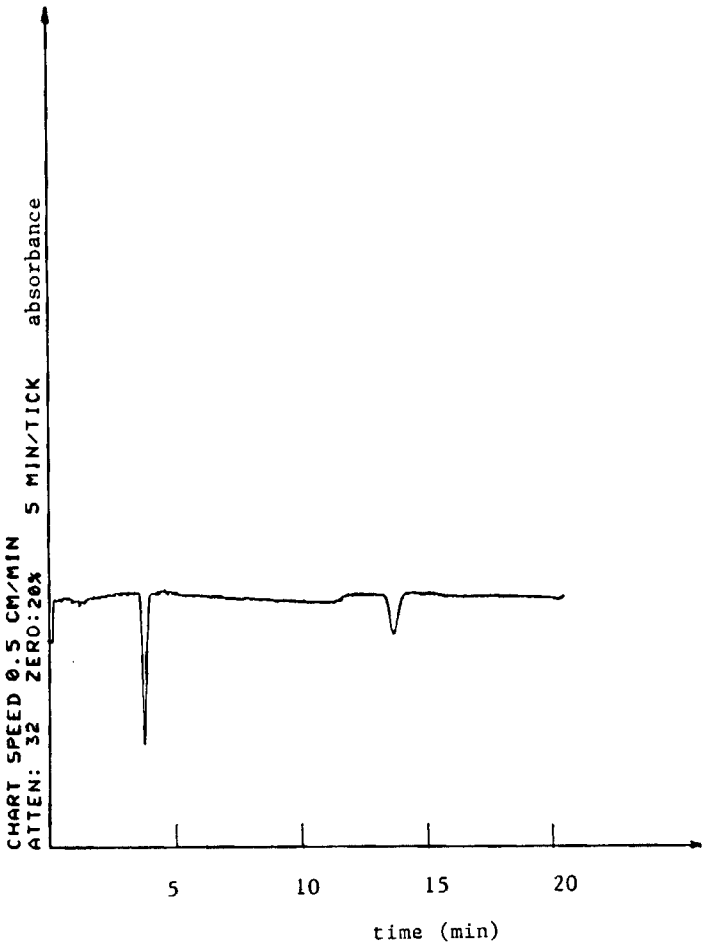

Figure 4 -
Conditions : as in figure 2.
Sample : 30 µl of $NaNO_3$ - 10.00 ppm.

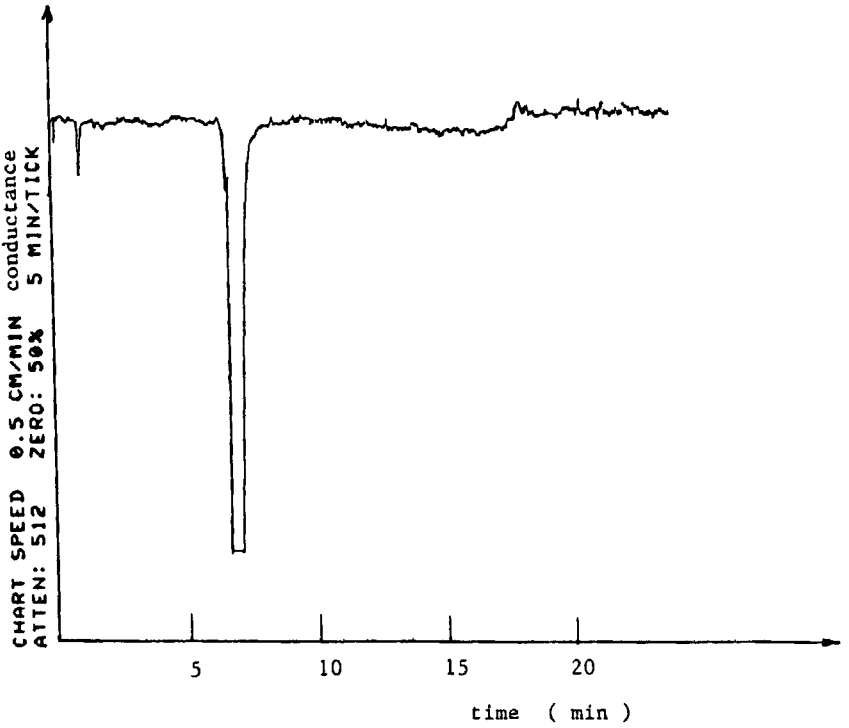

Figure 5 -
Conditions:Column RP C-18 , 10 μ m;IIR = octylamine (OCT)-chloride 0.0050 M; flow 2.0 ml/min; detection : conductometric .
Sample : NaCl 10.00 ppm (100 μ l).

of dilution and the injection peak,even if always of negative sign,has a very smaller area. When NaCl concentration of the injected sample (100 microliters) is 500.0 ppm, the conductance contribution will prevail on dilution and this will result in a wide positive injection peak (figure 6).

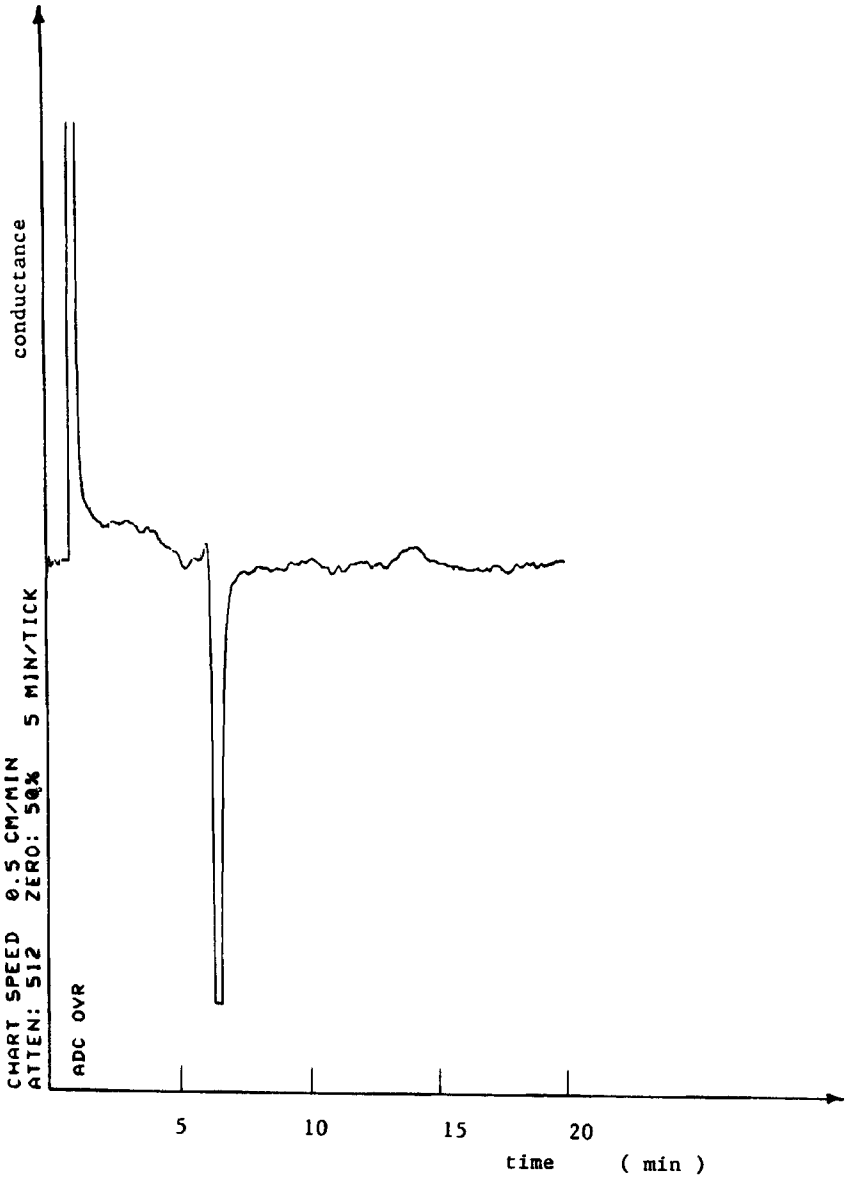

Figure 6 -
Conditions : as in figure 5.
Sample : NaCl 500.00 ppm (100 µ l).

As it concerns the addition of ions which are common with the eluent itself,it must be considered their concentration.If this is less than the eluent (by respect to which the baseline is recorded),the dilution effect will prevail.

If concentrations are equal,i.e. if for example 100.00 microliters of eluent itself are injected, no variation is observed at all.If concentration is higher, addition of both C^+ and A^-, independently or together, will shift the equilibrium (1) towards an increase of CA(ads).This process can proceed up to get a sort of "saturation", which depends on the number of disposable sita on the column, taking into account the effects of both steric restictions and electrostatic repulsion forces, acting between positive C^+ions in the IHP.This situation may be different for different IIR.

In the example above described concerning injection of NaCl in a system octylamine-chloride, the behaviour of unretained Na^+ ion was considered.As it concerns the anion Cl^-, common to the eluent ,it ,on the contrary, partecipates at some extent to the equilibrium (1),with a consequent increased amount of CA(ads);so when the injected amount of NaCl is 500 ppm, the system peak,even if still of negative sign ,shows (figure 6) a lower area than when water or more diluted solution are injected.

In turn,when the system is formed by OCT-salicylate (0.0050 M) ,UV detection (λ = 254 nm), an injection of salicylate ion in concentration ten folds higher than eluent's, acts in the same direction,but with more enhanced effect: the system peak not only decreases its area but it does become of positive sign .

These two behaviours are only apparently different:in both cases the addition of an ion common with the eluent causes a greater amount of CA to be adsorbed. Moreover the entity of this increase does depend on the specific properties of the IIR considered: it might be therefore suggested that OCT - chloride in the used experimental conditions of column and concentration (0.0050 M) is in a situation near to saturation, likely due to effects of ion size,solvatation and charges distribution.So that,nevertheless for addition of Cl^-, the amount of CA(ads) somehow increases,the effect of dilution prevails and the peak system is still of negative sign.

On the contrary,for OCT- salicylate,the concentration of 0.0050 M is lower than that which determines saturation.For injections in this system of salicylate as sodium salt 0.0500 M (figure 7) or octylamine 0.0500 M (figure 8) or both (as octylamine salicylate 0.0500 M) (figure 9), always positive system peaks are obtained. It is worth to be mentioned that

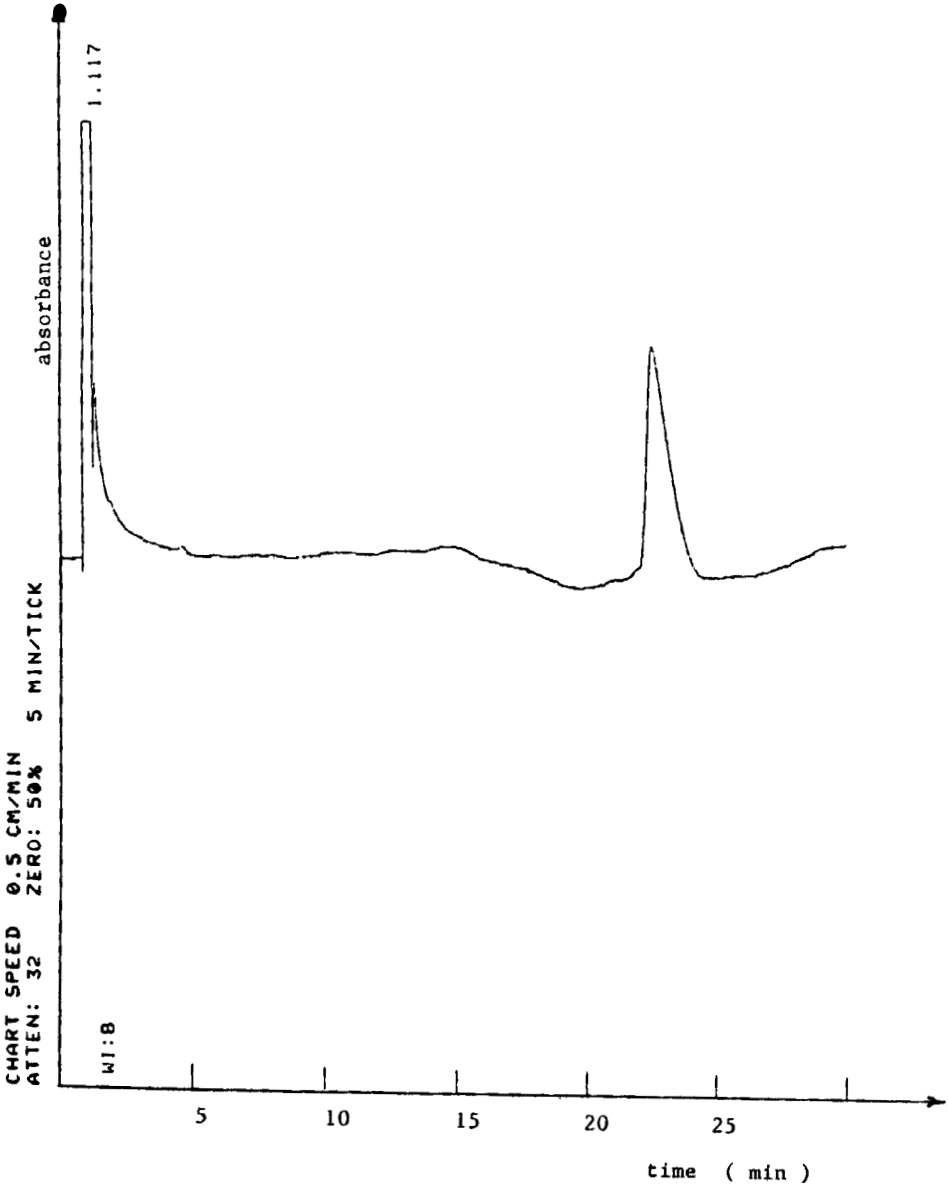

Figure 7 -
Conditions:Column RP C-18 , 5 µm spherical;IIR = octylamine (OCT)-salicylate 0.0050 M; flow 2.0 ml/min; detection : UV (254 nm).
Sample : Sodium salicylate 0.0500 M (100 µl).

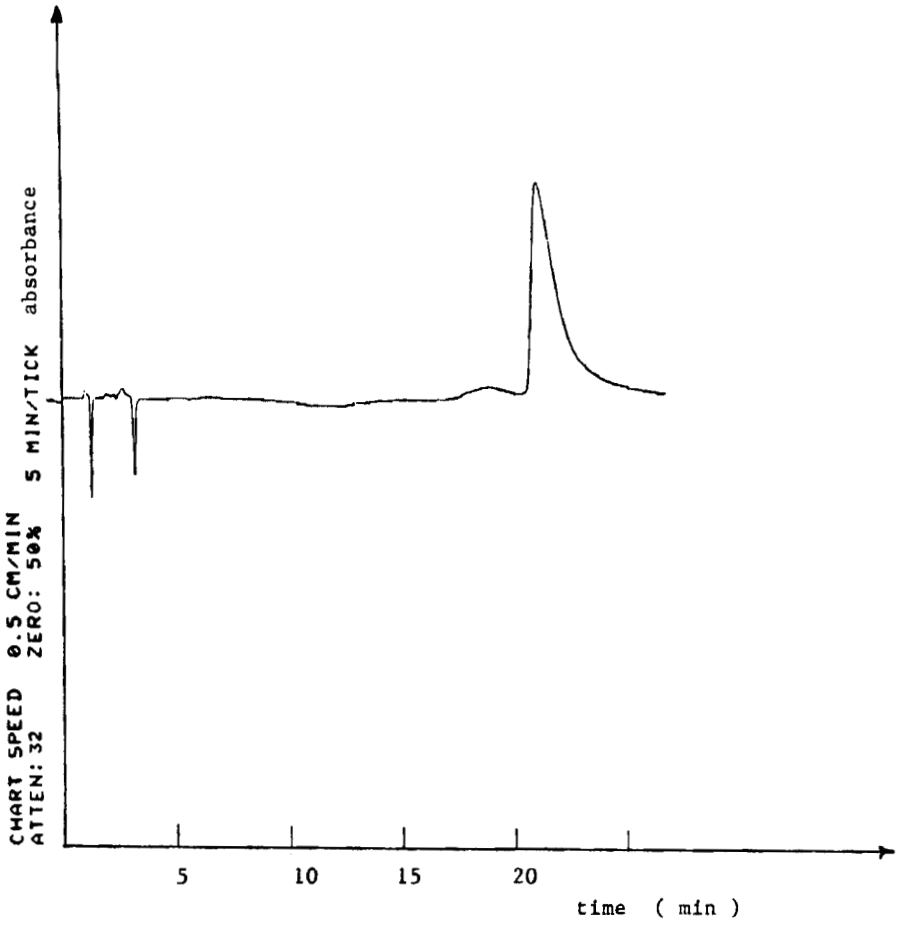

Figure 8 -
Conditions : as in figure 7.
Sample : octylamine 0.0500 M (100 µl).

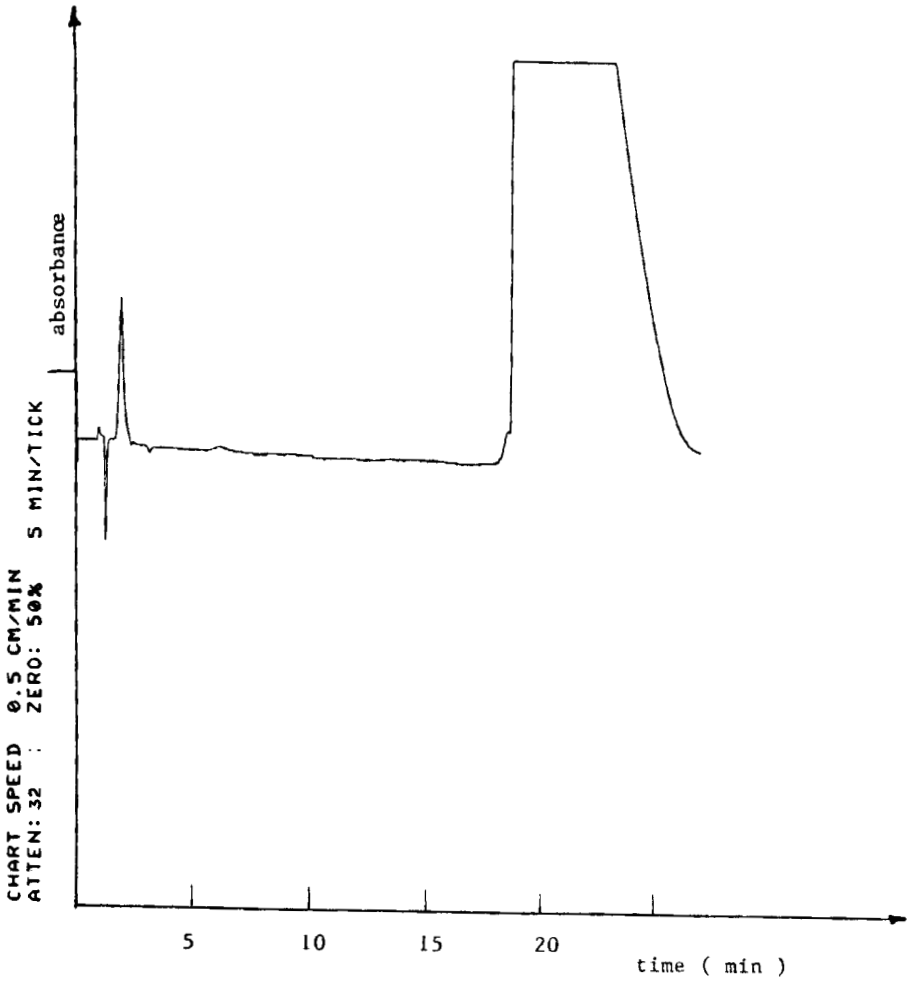

Figure 9 -
Conditions : as in figure 7.
Sample : octylamine salicylate 0.0500 M (100 µl).

addition of ions common with eluent is the only case which was envisaged able to determine positive peak systems.In figure 7 (injection of sodium salicylate 0.0500 M,UV detection at λ = 254 nm),besides the mentioned positive system peak,also a positive injection peak can be observed.This is due to a part of salicylate ions which being in excess by respect to available octylamine (equilibrium (1)) are unretainable and contribute , taking into account both concentration and molar absorptivity, to the injection peak with a positive contribution.

This experiment confirms the proposed mechanism: the anions A^- can not be retained,unless they have the possibility to form with the hydrophobic cation a CA(ads) species adsorbable.

The excess of octylamine (same excess) likely behaves the same but,due to its ε value lower than eluent's, it does not contribute to the injection peak in spectrophotometric detection.

Figure 10 shows the effect of the injection of 10 microliters of a solution of NaOH 0.0100 M in the OCT- salicylate 0.0050 M system, UV detection.The result may be explained by assuming that OH^- ions engage octylammmonium ions in acid-base equilibria,competing with equilibrium (1) which shifts towards desorption of some CA(ads); the result is a positive injection

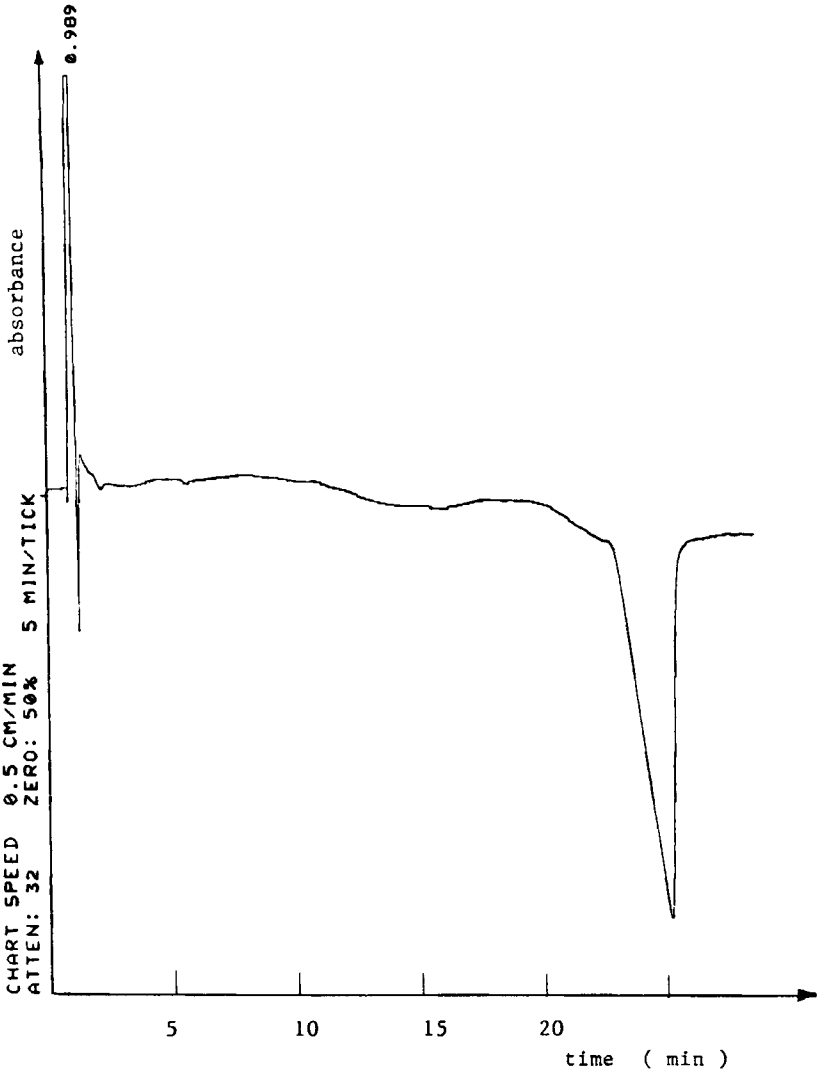

Figure 10 -
Conditions : as in figure 7.
Sample : NaOH 0.0100 M (100µ 1).

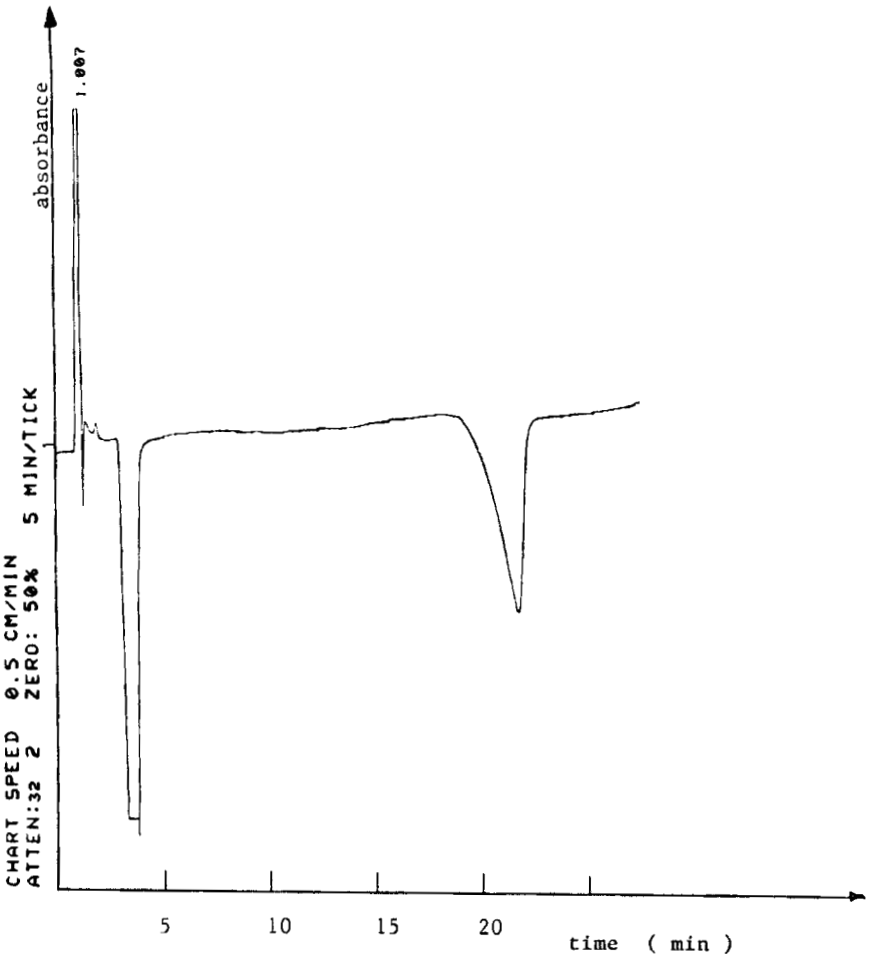

Figure 11 -
Conditions: as in figure 7.
Sample : $NaNO_3$ 0.0500 M (100 µ 1),prepared in octylamine salicylate 0.0050 M.

peak (salicylate displaced and unretained) and a corresponding negative system peak (lower amount of CA(ads) adsorbed).

Figure 11 shows a chromatogram obtained by addition to the same OCT-salicylate system (UV detection) of 30 μ l of $NaNO_3$ 0.050 M,prepared in the eluent itself (OCT-salicylate 0.0050 M), to avoid effect of dilution .It can be noticed the presence of three peaks: the injection peak which has positive sign, due to the salicylate ions displaced by nitrates in strong excess; the second one has negative sign (decrease of absorbance by respect to the eluent) and is due to nitrates.The third is the peak system and, as expected, shows negative sign.

CONCLUSIONS

The results of these experiences were collected in table I, in which injection peak and system peak for the investigated systems are listed.

For comparison,are also reported some retention times for nitrate ions, evaluated in the same chromatographic conditions.

It may be concluded that injection and system peaks do depend on the overall of the chromatographic conditions, in particular way on the characteristics and properties of the IIR, on the silica base column and the kind of detector.

As it concerns injection peak, its time was compared with the retention time corresponding to the void volume;it has moreover to be pointed out that it does depend not only on the apolar column, but on the whole column-IIR system. By comparing the results of table I ,it can be observed that time shown by injection peak when TBA-chloride is employed as IIR (for the same C-18 column)is greater than that for OCT-chloride. Such a difference can be explained in terms of greater porosity of TBA-chloride, which property is generally correlated with a greater void volume.

In other words, all parameters which cause higher retention time in the IIR RP HPLC chromatographic technique determine also higher times for the peak system.So, for example, eluent being OCT salicylate 0.0050 M in both case, higher times for the system peaks are shown for a C-18 column with 5 μm spherical packing,with respect to a C-18 10 μm column.

It follows that, such as the entity and the effect of varied chromatographic conditions on the IIR RP HPLC chromatographic technique can hardly foreseen a priori, the same holds for the system peaks.Almost a run of water injection will be necessary.

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