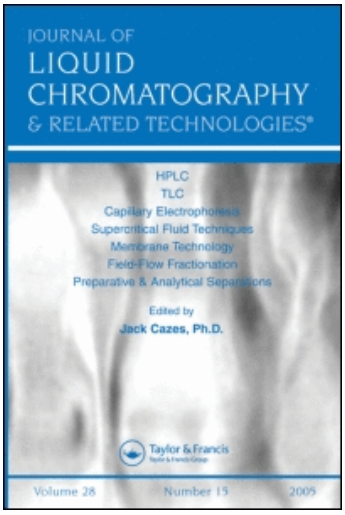


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# Indirect Detection in High-Performance Liquid Chromatography

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# INDIRECT DETECTION IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Indirect detection in high-performance liquid chromatography is described. It is classified into three categories according to the interaction between the analyte and the visualization agent. Sensitivity of indirect detection, detectors and estimation of the signal intensity of the induced peak are discussed. Some applications are demonstrated.

## INTRODUCTION

Flame ionization detectors (FID) or mass spectrometers have been commonly employed in gas chromatography (GC). These detectors are universal, sensitive or versatile, which is one of the reasons why GC has been widely utilized as a separation tool. Mass spectrometers are promising as the versatile detector for high-performance liquid chromatography (HPLC), and various ionization techniques and interfaces have been investigated for coupling HPLC and mass spectrometry (MS). However, the state of the art of HPLC/MS is still far from that of GC/MS.

Refractive index detectors have been employed as the universal detector in HPLC in spite of their poor sensitivity and inapplicability to gradient elution.

Indirect detection is expected to be universal and sensitive in HPLC. It allows detection of ions (or ionic species) as well as nonionic species (1). Indirect UV (ultra-violet) detection in ion chromatography, which was initiated by Small and Miller (2), is one of the most successful application of indirect detection. Indirect detection is defined in this paper as the case that the analyte is transparent and visualized by the variation of the background or by the detection of the separate species produced by postcolumn interaction.



## CLASSIFICATION OF INDIRECT DETECTION

Indirect detection can be classified into three categories accoding to the type of the interaction between analytes and visualization agents: ① mobile-phase induced; ② postcolumn interaction; ③ no interaction (dilution).

In the first case, the mobile phase component visualizes analytes via interaction such as displacement, ion-pair formation, perturbation of partitioning process, etc. In this case, the mobile phase component maintains a background, and the analytes are detected owing to the variation of the background. Indirect UV detection in ion chromatography is an example of this case, in which analyte ions displace the mobile phase ions in order to maintain neutrality of the charge in the analyte band (2).

When the analyte perturbs the dynamic partition process of the mobile phase component (visualization agent), variation of the concentration of the latter species over the separation column takes place. Detection of ionic species in ion-pair chroma-

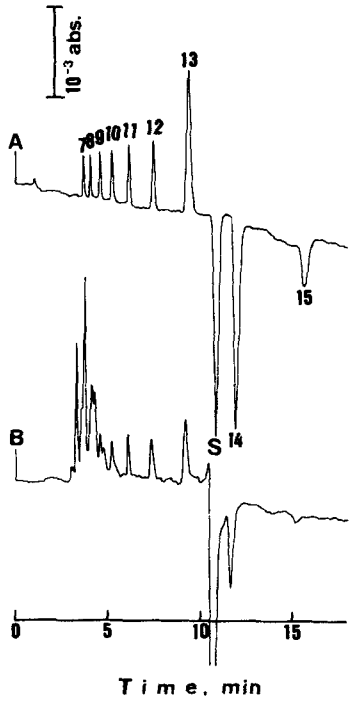


Figure 1 Indirect UV detection of an artificial mixture of hydrocarbons and components in kerosine. Column:ODS, 150 x 0.34 mm i.d. Mobile phase:methanol including $1.2 \times 10^{-4}$ M benzo[a]pyrene. Flow rate:4.2 $\mu$l/min. Sample:0.5 % (V/V) each; the numbers correspond to the carbon numbers of straight-chain hydrocarbons (A); and 6.7 % (V/V) kerosine (B). Wavelength of UV detection:300 nm. (Reproduced from reference 3 with permission from Elsevier Science Publishers BV).

tography or uncharged species in reversed-phase HPLC are also involved in this case.

Figure 1 demonstrates the indirect UV detection of an artificial mixture of hydrocarbons (heptane to pentadecane) and components in kerosine using benzo[a]pyrene as the visualization agent (3). The retention time of the system peak coincides with that of benzo[a]pyrene. The analytes eluted before the system peak give positive peaks, while the analytes eluted after the

systeme peak give negative peaks. The analytes eluted close to the system peak give larger signals.

In the second case, postcolumn interaction generates the separate species corresponding to the analytes, which are subjected to adequate detection. In this case, the visualization agents are loaded on the postcolumn, and the mobile phase component does not generally provide any background.

Postcolumn ion-replacement in ion chromatography (4) and enzyme reaction (5) can be involved in the second case. An example of the former case is dual-column ion chromatography with ion-replacement. When the analyte ion passes through the postsuppressor ion-replacement column (or membrane), it coelutes with the reagent ion with the opposite charge, or displaces the reagent ion with the same charge. When the reagent ion responds to the detector, the analyte ion can be indirectly detected.

Figure 2 demonstrates the indirect UV detection of an artificial mixture of monovalent cations via postsuppressor ion replacement (6). $\beta$-naphthalenesulphonate ($\beta$NS) is used as the chromophoric ion. The analyte cations coelute with $\beta$NS from the ion-replacement column and are detected at 225 nm. The concentration of each analyte is 1 mM, corresponding to 0.14 to 0.78 ng of the injected amounts. The detection limits at S/N=2 were ca. 0.01 mM, and the mass detection limits were ca. 0.2 pmoles.

Various organic compounds are indirectly detected after postcolumn enzyme reaction. The enzyme reaction commonly produces hydrogen peroxide and a reduced form of $\beta$-nicotinamide-adenine dinucleotide (NAD), NADH, which are subjected to adequate detection. This type reaction is generally specific and sensitive.

Figure 3 demonstrates the fluorometric detection of an artificial mixture of bile acids (7). The 3$\alpha$-hydroxy group in bile acids is oxidized to a keto group by the enzyme reaction, while NAD is reduced to NADH. Hydroxysteroid dehydrogenase (HSD) immobilized onto the porous glass beads is used in Figure 3. The detection limits at S/N=2 were 0.13-0.28 pmoles.

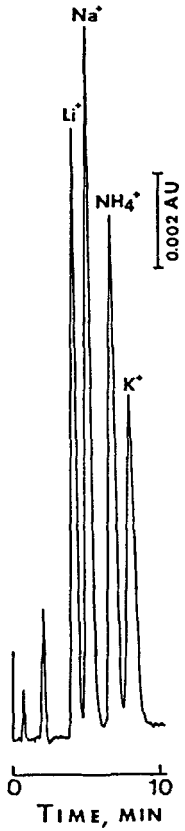


Figure 2 Indirect UV detection of an artificial mixture of monovalent cations using $\beta$NS as the chromophoric ion. Column:IC-Cation (50 x 0.35 mm i.d.); suppressor ($OH^-$-form; 40 x 0.35 mm i.d.); ion-replacement column ($\beta$NS-form; 30 x 0.35 mm i.d.). Eluent:2 mM nitric acid. Flow rate:2.8 $\mu$l/min. Samples: 1 mM each. Wavelength of UV detection:225 nm. (Reproduced from reference 6 with permission from Vieweg Publishing).

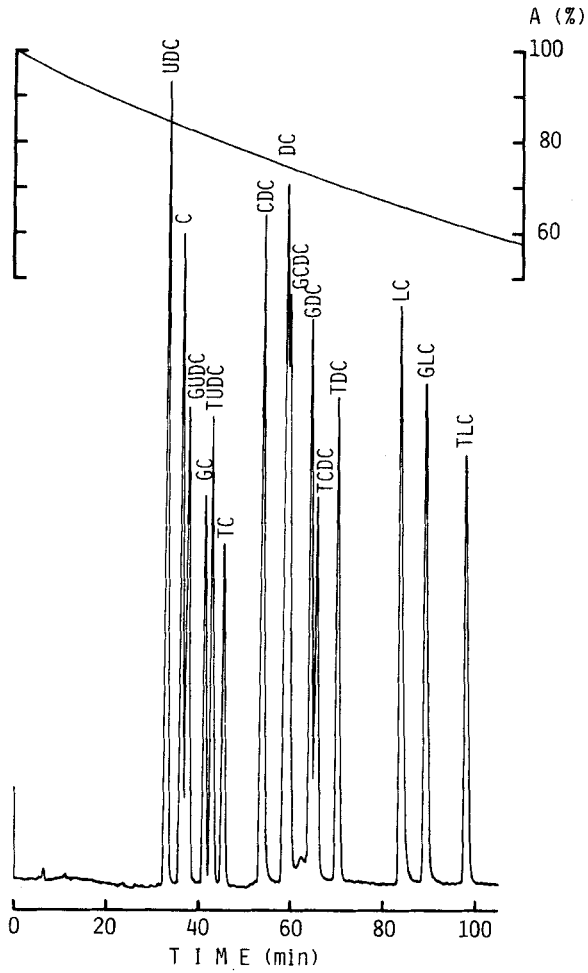


Figure 3 Indirect fluorometric detection of an artificial mixture of bile acids.
Column:ODS, 200 x 0.26 mm i.d. Guard column:ODS, 5 x 0.20 mm i.d. Mobile phase:60 mM phosphate(pH=9.8)/60 mM phosphate(pH=8.9) containing 18 mM NAD/acetonitrile(70:10:20)(A); 60 mM phosphate (pH=9.5)/60 mM phosphate(pH=8.9) containing NAD/acetonitrile (30:10:60)(B); gradient profile as indicated. Postcolumn:HSD, 20 x 0.34 mm i.d. Wavelength:excitation, 365 nm; emission, 470 nm. Samples:19-23 ng each. (Reproduced from reference 7 with permission from Elsevier Science Publishers BV).

Some cases of chemiluminescence detection can be involved in the second case.

In the third case, no interaction between the analyte and the visualization agent is involved, i.e., the concentration of the visualization agent is varied by dilution with the analyte. Therefore, the sensitivity of this category is poor, as discussed later.

## DETECTORS

Various detectors have been used in indirect detection, involving UV absorption, fluorescence, electrical conductivity, flame emission, etc. All the detectors for HPLC can be basically employed for indirect detection, among which the UV detector has been most commonly used. When the background is maintained to some degree, e.g., the first and third cases, the dynamic reserve plays an important role in sensitivity. The dynamic reseve is defiend as the ratio of the background level to its noise level (8). The larger the dynamic reserve, the smaller detection limit is achieved. The typical dynamic reserves are $2\times10^4$ for UV detection (9), $5\times10^3$ for double-beam fluorometric detection with high-frequency modulation (8,10), $2.5\times10^7$ for optical activity detection (11), and $10^6$ for refractive index detection. The latter two detectors work well as a universal detector because they provide larger dynamic reserves.

## DETECTION LIMIT

The detection limit at S/N=n achieved by indirect detection is given by the following equation (9):

$$C_{lim} = \frac{nC_m}{D_R(R+C_mV_s-RC_mV_m)+C_m(RV_m-V_s)} \qquad (1)$$

where $C_{lim}$ is the detection limit of the detector, $C_m$ is the concentration of the visualization agent, $D_R$ is the dynamic reserve, R is the displacement ratio (defined as the number of the visualization agent species which are displaced by one analyte species), $V_m$ and $V_s$ are molar volumes of the visualization agent and the analyte, respectively. This equation was derived by assuming that the mixing volume is additive.

In case the valence of the analyte ion is equal to that of the mobile phase ion in ion chromatography, Eq. 1 is rewritten by substituting R=1 and by assuming that $V_m=V_s$:

$$C_{lim} = nC_m/D_R \qquad (2)$$

Eq. 2 indicates that the lowest detection limit is achieved with the lowest concentation of the visualization agent if the dynamic reserve and the retention volume of the analyte ions are maintained. This encourages us to use a visualization agent having a high response factor to the employed detector. However, a stationary phase with a lower ion-exchange capacity, which allows elution of the analyte ions in a reasonable time, must be used, otherwise the improvement of the detection limit will be cancelled.

When only dilution is involved, Eq. 1 is rewritten by substituting R=0:

$$C_{lim} = \frac{n}{(D_R-1)V_s} \fallingdotseq \frac{n}{D_R V_s} \qquad (3)$$

In this case, the detection limit is independent of the concentration of the visualization agent, but is inversely proportional to the dynamic reserve. Therefore, the sensitivity achieved in this case is much poorer than that achieved according to Eq. 2.

## SIGNAL INTENSITY OF INDUCED PEAK

The signal intenisty of the induced peak in ion chromatography is easily estimated from the valences of the visualization

agent and the analyte ions as well as the response factor of these species to the employed detector. The peak direction is generally negative in indirect detection in ion chromatography.

On the contrary, it is more difficult to estimate the signal intensity in ion-pair or reversed-phase modes. The signal intensity in indirect detection has been discussed by Schill and Crommen. They derived equations for both charged and uncharged analytes(1):

$$\Delta C_k = \theta \cdot \frac{\alpha_s}{1-\alpha_s} \qquad (4)$$

$$\Delta C_k = -(1-\theta) \cdot \frac{\alpha_s}{1-\alpha_s} \qquad (5)$$

where $\Delta C_k$ is the change of the concentration of the mobile phase component k, $\theta$ is the fractional coverage of the solid phase by k, and $\alpha_s$ is the retention of the analyte j relative to k, i.e., $\alpha_s = k_j'/k_k'$. Eq.4 is for uncharged analytes, while Eq.5 is valid when j and k have opposite charges.

The authors derived an empirical equation for the reversed-phase HPLC (12):

$$S = FC_{aa}W_{bb}|c| \cdot \frac{\phi}{\phi+1} \cdot \frac{k_a'(k_b'+1)}{|k_a'-k_b'|} \qquad (6)$$

where S is the peak area of the induced peak, F is a response factor, $C_{aa}$ is the concentration of the visualization agent, $W_{bb}$ is the amount of the analyte injected, c is the coefficient accounting for a degree of the variation of the capacity factor of the visualization agent, $\phi$ is the phase ratio, $k_a'$ and $k_b'$ are the capacity factors of the visualization agent and the analyte, respectively. Eq.6 indicates that the peak area of the analyte increases with increasing $k_a'$ and $k_b'$ and decreasing a difference bewteen $k_a'$ and $k_b'$. This means that the detection limit of the analyte can be improved by the careful selection of the operating conditions.

The peak directions in ion-pair and reversed-phase modes are complex. The directions are given from Eqs.4 and 5.

In conclusion, indirect detection in HPLC provides high sensitivity by the careful selection of the mobile phase conditions or the type of the postcolumn interaction. Detectors with a high dynamic reserve shoud be developed for universal detection in HPLC.

## REFERENCES

1. G. Schill and J. Crommen, Trends Anal. Chem., 6, 111(1987).
2. H. Small and T.E. Miller, Jr., Anal. Chem., 54, 462(1982).
3. T. Takeuchi and D. Ishii, J. Chromatogr., 396, 149(1987).
4. S.W. Downey and G.M. Hieftje, Anal. Chim. Acta, 153, 1(1983).
5. S. Okuyama, N. Kokubun, S. Higashidate, D. Umemura and Y. Hirata, Chem. Lett., 1443(1979).
6. T. Takeuchi, E. Suzuki and D. Ishii, Chromatographia, submitted for publication.
7. D. Ishii, S. Murata and T. Takeuchi, J. Chromatogr., 282, 569 (1983).
8. S. Mho and E.S. Yeung, Anal. Chem., 57, 2253(1985).
9. T. Takeuchi and D. Ishii, J. Chromatogr., 393, 419(1987).
10. T. Takeuchi and E.S. Yeung, J. Chromatogr., 366, 145(1986).
11. D.R. Bobbitt and E.S. Yeung, Anal. Chem., 56, 1577(1984).
12. T. Takeuchi, S. Watanabe, K. Murase and D. Ishii, Chromatographia, in press(#2, 1988).