Waters
Lab Highlights
An Internal Communication of Applications and Techniques
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## ADVANCES IN PAIRED-ION CHROMATOGRAPHY

# II. UV-VISUALIZATION LIQUID CHROMATOGRAPHY: A NEW TECHNIQUE FOR DETECTION AT 254 NM OF NON-UV-ABSORBERS

The fundamental concepts of the ion-interaction model (IIM) for reversed-phase, paired-ion HPLC were presented in the previous Lab Highlihgt of this series. Based on the IIM, the prediction was made that non-UV-absorbing solutes could be detected at 254 nm if a UV-absorbing paired-ion reagent was used (1). This prediction was soon verified at Waters Associates (2,3) and the technique was named "UV-Visualization liquid chromatography."

The UV-visualization technique works because the IIM correctly states that adsorbed paired-ion reagent will coelute with the sample. By adding a moderate concentration (≃ 0.2 mM) of a UV-absorbing reagent to a reversed-phase eluent, a background UV absorbance of around 0.5 AU is produced. This can be electrically offset without difficulty. Coelution of reagent with a non-UV-absorbing sample will produce a UV response which is above (or below) the background level, and this allows for detection.

A typical example of UV-Visualization is the separation and detection (UV) of four alkylsulfonates shown in Figure 1. Some unusual features of this chromatogram should be noted. First, there are four positive peaks (for the four alkylsulfonate solutes) plus a negative peak preceding the first of the four peaks. This negative peak occurs at the retention time which the reagent has when injected as a sample. Second, the response for the alkylsulfonates is not proportional to the molar amounts injected. In Figure 1A, 0.5 µg of each alkylsulfonate was injected. This corresponds to injection of 2.92, 2.70, 2.51 and 2.35 nanomoles respectively of pentane-, hexane-, heptane- and octanesulfonate. The peaks resulting from this relatively equimolar mixture are clearly not equal in area or height. Although this finding has not yet been explained, it does not limit the utility of the UV-Visualization method.

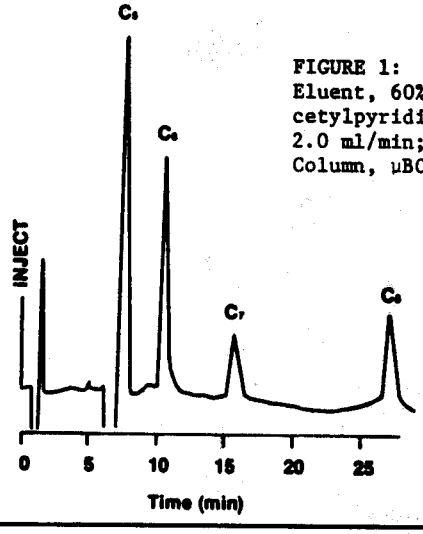


FIGURE 1: UV-Visualization of Alkylsulfonates. Eluent, 60% MeoH: 40% $H_2O$ with 0.2 mM cetylpyridinium chloride (CPI); flow rate 2.0 ml/min; detector, 254 nm at 0.05 AUFS, Column, µBONDAPAK™ $C_{18}$.

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Applications of UV-Visualization chromatography are gradually appearing in the literature, as summarized in Table I. The method is appealing because of its great simplicity. Both pre-column derivatization and post-column reaction have been used to enhance the detectability of non-UV-absorbers, but these methods are considerably less convenient than UV-visualization. For this reason, increasing application of this technique should be seen in the future.

TABLE I

APPLICATIONS OF UV-VISUALIZATION CHROMATOGRAPHY*

| APPLICATION | REAGENT | ELUENT | COLUMN | REF. |
|---|---|---|---|---|
| Alkyl Sulfonates ($C_5$, $C_6$, $C_7$, $C_8$) | Cetylpyridinium Chloride @ 0.2 mM | 60% MeOH 40% $H_2O$ | µBONDAPAK™ $C_{18}$ | 2, 4 |
| Alkyl Sulfonates ($C_5$,$C_6$, $C_7$, $C_8$) | Phenethylamine @ 5 mM | 35% MeOH 65% $H_2O$ pH 3 | µBONDAPAK™ $C_{18}$ | 3 |
| Octylamine | p-Ethylbenzene-sulfonic Acid @ 5 mM | 50% MeOH 50% $H_2O$ pH 3 | µBONDAPAK™ $C_{18}$ | 3 |
| Bile Acids | Hyamine 1622 @ 0.09% (w/v) | 75% MeOH 25% $H_2O$ 2mM $(NH_4)_2HPO_4$ pH 7.5 | Chromegabond $C_{18}$ | 5 |
| Ionic and Amphoteric Surfactants | Sodium Decylbenzene Sulfonate @ 4.2 mM | 90% MeOH 10% $H_2O$ pH 4 | µBONDAPAK™ $C_{18}$ | 6 |
| Carboxylic Acids, Quaternary Ammonium Salts | 1-Phenethyl-2-picolinium bromide @ 0.3 mM | Acetate buffer pH 4.6 | µBONDAPAK™ Phenyl | 7 |
| Alkyl Sulfates, Alkyl Sulfonates, Alkyl Amines Amino Acids, Dipeptides | Naphthalene-2-Sulfonate @ 0.4 mM | 0.05 M $H_3PO_4$ aqueous | µBONDAPAK™ Phenyl | 7 |

* Detection is at 254 nm for all applications

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