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OPTIMUM CHROMATOGRAPHIC CONDITION FOR 2,6,-DI-t-BUTYL-4-PYRAZINYLAMINOPHENOL ON RP-HPLC: METAL INFLUENCE ON ITS ELUTION PROFILE

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ABSTRACT

The optimization of chromatographic conditions for the determination of 2,6-di-t-butyl-4-pyrazinylaminophenol(DBAP) on reversed-phase HPLC with an aqueous-organic mobile phase were investigated. Undesirable peak shape, of which the quinone form(QF) was produced by the decomposition of DBAP in a stainless steel column, was observed on both of RPC packings containing small and large amounts of metal impurities. Although this conversion reaction was suppressed by washing the ODS gels with a 1 mM EDTA solution, the decomposition of DBAP in the column was still taking place. Concluding that the contamination of metal impurities on the column packing occurs continuously. The use of inert columns such as peek(polyether-ether-ketone), glass-lining columns but except a titan column, packed a high purity RPC packing material which was washed with the EDTA solution prior to use, led to the quantitative result of DBAP in the inert chromatographic system.

INTRODUCTION

Reversed-phase HPLC has been widely employing for determinations of various compounds by means of giving high speed and excellent resolutions. One of the defects on RP-HPLC, however, is to accompany decomposition reactions of many samples induced by water and metal ions in an LC column with an aqueous mobile phase. For instance, Horvath and his coworkers have reported that hydroquinone and their derivatives were converted to their corresponding quinone forms by the redox reaction (1,2).

The reasons for the introduction of the redox reaction are as follows;

- (1) Self-oxidation by water or oxygen
- (2) Metal ions from chromatographic system employed, column packings and column materials

The metal-ion effect on chromatographic behavior for various substances has been discussed by many researchers, for example, ferroxamine(2), acetylacetone (3,4,5), 8-quinolinol(5) and amines(6). They have been emphasized that the pronounced effects of metal ion on their chromatograms were observed on the packings contained large amounts of metal impurities, but less on the column packings made from high purity silica gel(3,4,5).

We have been observing the redox reaction of a hydroquinone analogue using packings produced by high purity silica gel after washing by EDTA solution.

We report the optimum condition of the determination of 2,6-di-t-butyl-4-pyrazinylaminophenol (DBAP) on RPC using high purity ODS gel.

EXPERIMENTAL

Materials

TSKgel ODS-80T_M (5 μ m, TOSOH CO., Japan) and LiChrosorb RP-18 (10 μ m, E. Merck, Germany) packed into stainless steel, titan and peek columns (250mmL x 4.6mmID, purchased from Sugiyama CO., Tokyo) and a glass-lining column (250 mmL x 4mmID from GL Science LTD., Tokyo).

DBAP was home-made from 2,6-di-t-butyl-1,4-benzoquinone and aminopyrazine (the structures of DBAP and QF were illustrated in FIGURE 1).

Mobile phases were used 75% (v/v) of acetonitrile in Milli-Q water for the analysis of DBAP and 60% (v/v) of methanol in 0.5% sodium acetate aqueous solution for acetylacetone (AA) and nitronaphthalene (NN) measurement, respectively.

75% (v/v) of acetonitrile containing 1mM EDTA was employed for column washing at a flow rate of 0.5ml/min. DBAP was solved in HPLC-grade acetonitrile prior to use.

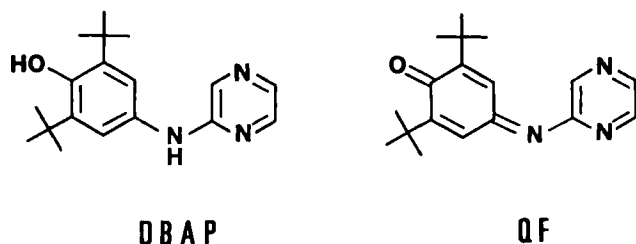


FIGURE 1 Structures of DBAP and QF

Chromatographic system

To estimate the metal influence in the column, an inert system equipped with a CCPM pump (resin model, TOSOH CO., Tokyo), peek tube (0.25mm ID, from Uppchurch Scientific Inc., USA), an injection valve (resin model 7125, Rheodyne) and a solvent degasser (a model SD-8012, TOSOH) for removing soluble oxygen in mobile phases. The samples were monitored at 285nm (for DBAP and QF) and 254nm (AA and NN) with a UV detector (a model UV-8010 TOSOH) was employed.

RESULTS AND DISCUSSION

FIGURE 2 shows the chromatogram of DBAP on TSK-gel ODS-80T_M (high purity ODS gel) and LiChrosorb RP-18 (contained large amounts of metal impurities) packed in the stainless steel columns using the inert system. Two peaks corresponding to DBAP and its quinone form (QF) which was produced by the oxidation reaction of DBAP at the inlet filter were observed on TSKgel ODS-80T_M.

a. TSKgel ODS-80_{T_M}

b. LiChrosorb RP-18

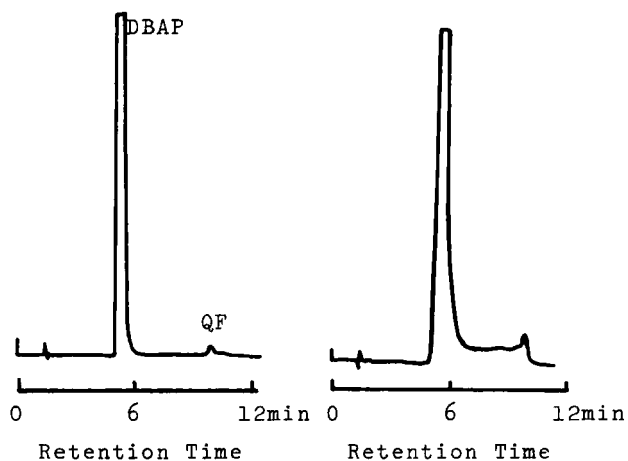


FIGURE 2 Comparison of the elution profile of DBAP on two commercial ODS column
 Column size; 250mmL x 4.6mmID, Mobile phase; 75%CH₃CN,
 Sample; DBAP (1mg/3mlCH₃CN, 20μl injection), Temp.; ambient
 Flow rate; 1.0 ml/min, Detector; UV 285 nm (0.08aufs).

On the other hand, a more complicated chromatogram was obtained on LiChrosorb RP-18 by means of the decomposition of DBAP on each part of the column bed (FIGURE 2-b).

The producibility of the diffused peak was achieved by injecting Fe³⁺ or Cu²⁺ solutions onto the ODS-80_{T_M} column (shown in FIGURE 3-a and b). These results indicate that the participation of Cu ion was stronger than Fe ion, as well as the previous result (1). Widespread metal ions on the packing material results in a broadened peak by the oxidation of DBAP in the whole column bed.

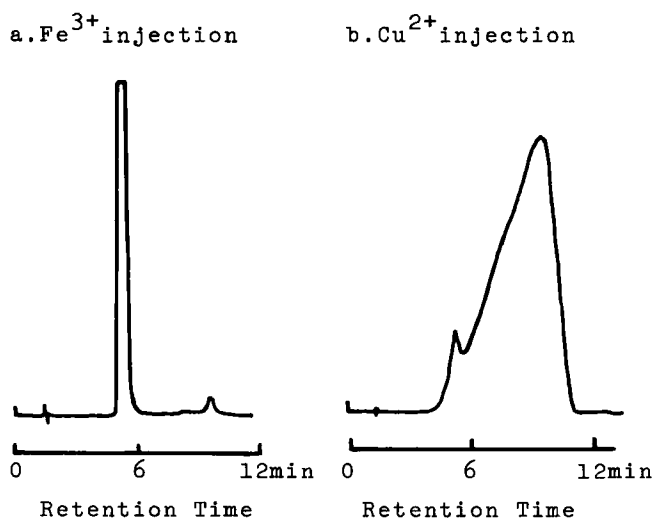


FIGURE 3 Effect of metal ion injection on DBAP elution profile
After injecting 500 μl portions of 1mM Fe^{3+} and Cu^{2+} solutions, DBAP was applied onto the ODS column.

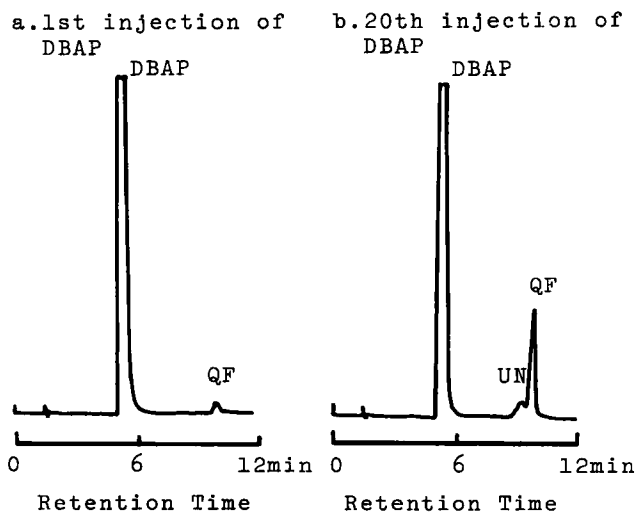


FIGURE 4 Influence of successive injection of DBAP on the ODS column
DBAP was introduced on the ODS column at 20min intervals.

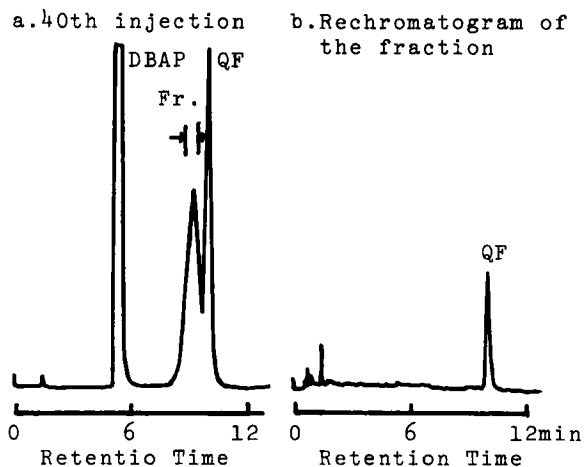


FIGURE 5 Rechromatogram of the fraction of the new peak on the 40th injection chromatogram. A 250 μ l portion of the fraticn was injected(b)

The successive injection of DBAP on ODS-80T_M at 20min intervals led to the appearance of an unknown peak in front of the QF peak(UN in FIGURE 4). The new peak was assigned to QF by the rechromatography of the fraction (shown in FIGURE 5) and the UV spectroscopic measurement of the fraction. It suggests that the oxidative reaction zone is gradually moving in the column during the analysis. In the case of measuring acetylacetone on this column ,however,normal peak shape was obtained as shown in FIGURE 6. Accordingly hydroquinones or their derivatives would be more appropriate samples to evaluate metal-ion effect in the chromatographic system.

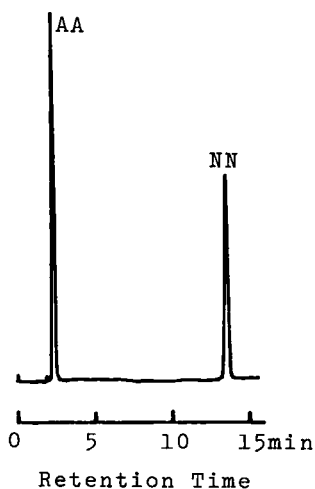


FIGURE 6 Chromatogram of chelating reagent on the ODS column in FIGURE 5-a.

Mobile phase; 60% CH_3CN in 0.5% $\text{CH}_3\text{CO}_2\text{Na}$, Detector; UV 254nm (0.16 au fs), Sample; acetylacetone (AA), Nitro-naphthalene (NN).

The oxidative region in the column could be completely removed by washing the column with the 1 mM EDTA solution in the reverse of the flow direction. However QF was appeared again on the chromatogram by the successive injection of DBAP. It would be seemed that the contamination of metal impurities on the packing material, especially leached from the fitting, continuously occurred.

FIGURE 7 shows the difference of the kind of column material, glass-lining (a), peek (b) and titan column (c), on the DBAP elution profile, respectively. Using the peek and glass-lining columns, no peak of QF was app-

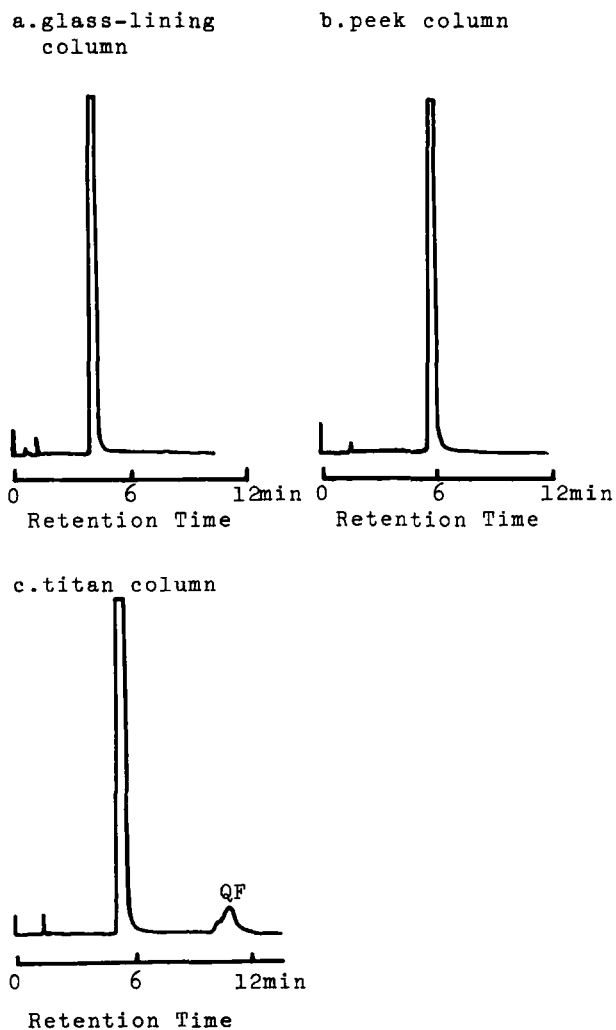


FIGURE 7 Comparison of column material as an inert column on the determination of DBAP
The chromatograms were obtained by the 20th injection of DBAP.

appeared on the chromatograms. On the contrary, the QF peak was observed using the titan column, which is well-known as an inert material. It would be implied that the titan column contains Fe or Cu ions on the fitting.

CONCLUSION

The optimization of the chromatographic condition for the determination of an oxidative sample, DBAP, on RPC was investigated. The influence of metal ion on the elution profile of DBAP was able to avoid by using high purity ODS column, which was washed with EDTA solution prior to analysis. As the redox reaction also depended on column materials employed, the contamination of the ODS column with metal ion continuously took place when a stainless steel column was used. Even if high purity ODS packing materials are utilized, the oxidative samples would be affected by metal ions leached from column fittings. It is concluded that the use of high purity ODS gel packed in an inert column such as peek and glass-lining column, and washed with an EDTA solution previously is recommended for the determination of oxidative samples.

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