

# High-performance liquid chromatography of mixtures of phenol or guaiacol and their reaction products with glyoxylic acid

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## HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF MIXTURES OF PHENOL OR GUAIACOL AND THEIR REACTION PRODUCTS WITH GLYOXYLIC ACID

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### SUMMARY

High-performance liquid chromatography has been used for a qualitative and quantitative analysis of reaction mixtures of phenol or guaiacol and glyoxylic acid in aqueous alkaline solutions. The reactants and the  $\alpha$ -hydroxyacetic acid derivatives of the starting aromatic compounds were analyzed using a strong cation-exchange resin (SCX) and an octadecylsilane modified silica (ODS) column, in combination with a refractive index detector or a fixed-wavelength UV detector. The application of a dilute aqueous solution of sulphuric acid as the eluent resulted in an almost identical elution pattern from the two columns. However, the selectivity for the aromatic compounds was found to be higher for the reversed-phase ODS column than for the ion-exchange SCX column. For both columns a separation mechanism based on hydrophobic interactions is suggested.

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### INTRODUCTION

Reactions of phenol or guaiacol (2-methoxyphenol) with glyoxylic acid result in mixtures of  $\alpha$ -hydroxyacetic acid derivatives of the starting aromatic compounds. Fig. 1 shows the reaction scheme for the system phenol-glyoxylic acid.

For reactions between guaiacol and glyoxylic acid an identical scheme holds. The most important products are D,L- $\alpha$ ,4-dihydroxybenzeneacetic acid (*p*-hydroxymandelic acid, phma) and D,L- $\alpha$ ,4-dihydroxy-3-methoxybenzeneacetic acid (vanillylmandelic acid, vma) for reactions of phenol and guaiacol, respectively. These mandelic acid derivatives are interesting intermediates for fine chemicals and pharmaceutical products, 4-hydroxybenzaldehyde, amoxicilline and atenolol from phma, vanillin and L-dopa from vma. For a complete description of the kinetics of these electrophilic substitution reactions of the rather expensive glyoxylic acid, an accurate method for a qualitative and quantitative analysis of the reaction mixtures is necessary. It is obvious that chromatographic separation techniques are very suitable for this purpose. Capillary gas-liquid chromatography (GLC) can be used for the analysis of mixtures of aromatic compounds as described by Schraufstetter *et al.*<sup>1</sup>. These

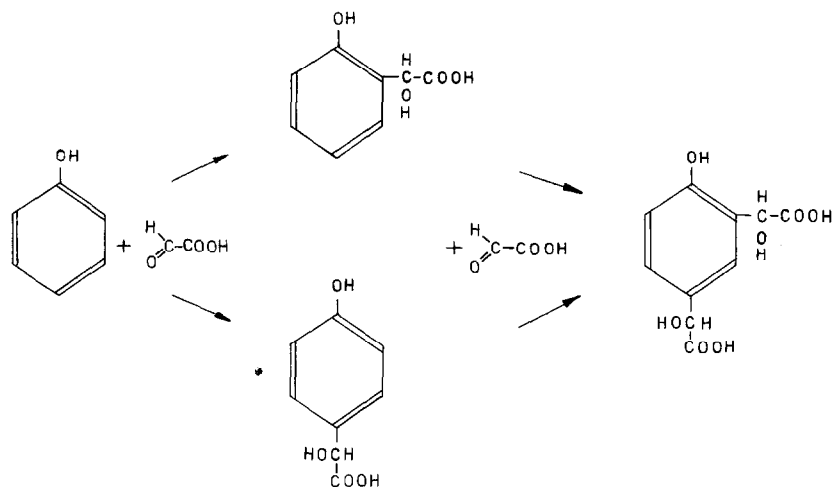


Fig. 1. Possible reactions in aqueous alkaline solutions of phenol and glyoxylic acid.

authors described the separation of mixtures of aromatic compounds including phenol and guaiacol. However, mixtures of phenol or guaiacol and their  $\alpha$ -hydroxyacetic acid derivatives were not investigated. The use of GLC for the analysis of this class of compounds is strongly hampered by a time-consuming pre-column derivatization necessary to increase the volatility and the thermal stability. Pre-column derivatization is not necessary when liquid chromatography is used. The chromatographic behaviour of aromatic compounds on columns packed with an anion-exchange resin was described by Nilsson and Samuelson<sup>2</sup> and by Jandera *et al.*<sup>3</sup>. In these studies the eluent was an aqueous solution of the sodium salts of various organic acids. However, the compressibility of the types of stationary phases investigated, at the eluent pressures used in modern liquid chromatography, is a serious disadvantage for the application of these materials in a separation column. Jandera *et al.*<sup>3</sup> and Jahangir and Samuelson<sup>4</sup> investigated the retention properties of non-ionic and sulphonated resins towards mixtures of aromatic compounds. These studies show that hydrophobic interactions predominate in the retention mechanism. The hydrophobic properties are strongly influenced by the functional groups in the resin. For sulphonated resins this results in a remarkable decrease in the retention volumes of aromatic compounds compared with those obtained on an unfunctionalized resin<sup>4</sup>.

Mixtures of derivatives of phenol were investigated with a reversed-phase system based on an octadecyl silica column<sup>5,6</sup>. Phosphoric acid-buffered acetonitrile-water mixtures<sup>5</sup> and solutions of formic acid in methanol-water mixtures<sup>6</sup> were used as the eluents. Because acid dissociation results in strong peak tailing, eluent acidification was necessary to suppress the dissociation of the acid groups in the compounds studied. The retention behaviour of many derivatives of phenol was reported<sup>6</sup>. However, the mandelic acid derivatives of phenol and guaiacol were not included.

The present paper reports a qualitative and quantitative high-performance liquid chromatographic (HPLC) analysis of mixtures formed during the reaction of phenol or guaiacol with glyoxylic acid in aqueous alkaline solutions.

## EXPERIMENTAL

Samples for chromatograms were taken from solutions obtained during the reaction of phenol or guaiacol with glyoxylic acid. These reactions were carried out in aqueous solutions of sodium hydroxide at a pH of 10.5 and a constant temperature between 25 and 60°C. The reactions were generally started with equimolar amounts of phenol or guaiacol and glyoxylic acid. The initial concentrations of phenol and guaiacol were 0.25 and 0.15 mol dm<sup>-3</sup>, respectively. The reactions were quenched immediately after sampling by adding the sample to an equal volume of an aqueous 1 mol dm<sup>-3</sup> hydrochloric acid solution. The resulting solution (pH < 1) was introduced in the chromatographic system without further handling.

The chromatographic system consisted of a Waters Type 6000A solvent-delivery system, a Rheodyne Type 7120 injection valve with a 10- $\mu$ l sample loop, a Jones Chromatography Type 7930 column heater, a Millipore Waters Type R410 refractive index detector, a Spectra Physics Type 8320 UV detector operating at 254 nm and a LDC/Milton Roy Type CI-10B computing integrator. Two columns were used: a 250 mm  $\times$  4.6 mm I.D. Lichroma stainless-steel tube slurry packed with a Benson Type BC-X8(H<sup>+</sup>) cation-exchange resin consisting of 7–10  $\mu$ m particles of a completely sulphonated styrene–divinylbenzene (8%) copolymer (strong cation exchange, SCX column) and a 150 mm  $\times$  4.6 mm I.D. Lichroma stainless-steel tube slurry packed with Merck LiChrosorb RP-18 consisting of 10- $\mu$ m particles of *n*-octadecylsilane-modified silica (octadecylsilane, ODS, column). The column temperatures were 85 and 50°C for the SCX and the ODS column, respectively. The eluent was an aqueous 5  $\cdot$  10<sup>-3</sup> mol dm<sup>-3</sup> sulphuric acid solution. Before use the eluent was filtered over a Millipore Type HAWP filter (0.45  $\mu$ m) and degassed with helium. Depending on the requirements, the samples were analyzed with one or more of the following combinations of columns and detection methods: SCX,RI; SCX,UV; ODS,RI and ODS,UV.

## RESULTS AND DISCUSSION

*Reaction mixtures of phenol and glyoxylic acid*

The chromatograms obtained with the column–detection combinations SCX,RI and SCX,UV are shown in the Figs. 2 and 3, respectively. Fig. 2 shows that a good quantification of phenol is possible with the combination SCX,RI. The elution of the main product of the reaction, phma, is visualized by the peak preceding that of phenol. The shoulder to the rear part of the elution peak of phma is probably the result of the elution of D,L- $\alpha$ ,2-dihydroxybenzeneacetic acid (*o*-hydroxymandelic acid, ohma). The shoulder to the tail of the elution peak of glyoxylic acid and the small peak immediately following are assigned to  $\alpha$ , $\alpha'$ ,4-trihydroxy-1,3-benzenediacetic acid (*o,p*-hydroxymandelic acid, ophma) and glycolic acid. Glycolic acid is one of the products of the Cannizzaro reaction of glyoxylic acid, which takes place to a small extent during the preparation of the starting solution. The other product of this disproportionation of glyoxylic acid is oxalic acid which is eluted in the injection peak. Since the absorbances of glyoxylic, glycolic and oxalic acid at 254 nm are negligible, the combination SCX,UV can be used to visualize only the elution zones of phenol and its condensation products with glyoxylic acid. Comparing Figs. 2 and

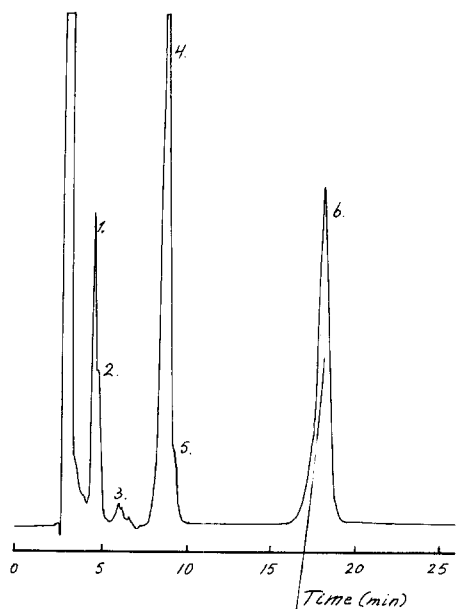


Fig. 2. Chromatogram of a sample from a reaction mixture of phenol and glyoxylic acid obtained with the column-detection combination SCX,RI. Analytical conditions as given in the Experimental section. Flow-rate of the eluent: 0.4 ml/min. peaks: 1 = glyoxylic acid; 2 = ophma; 3 = glycolic acid; 4 = phma; 5 = ohma; 6 = phenol.

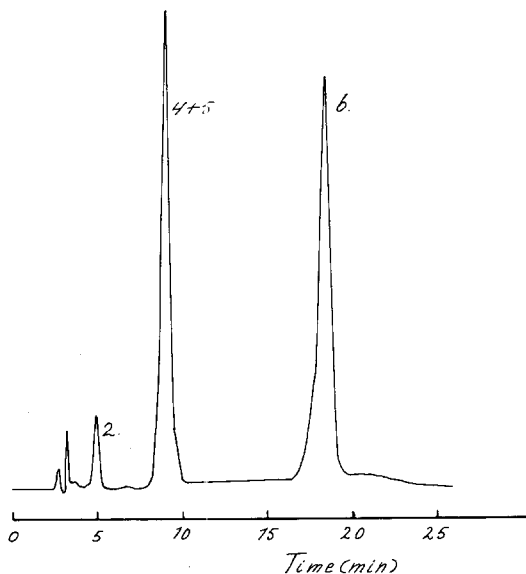


Fig. 3. Chromatogram of the same sample used in Fig. 2 obtained with the column-detection combination SCX,UV. Analytical conditions and peak numbering as in Fig. 2.

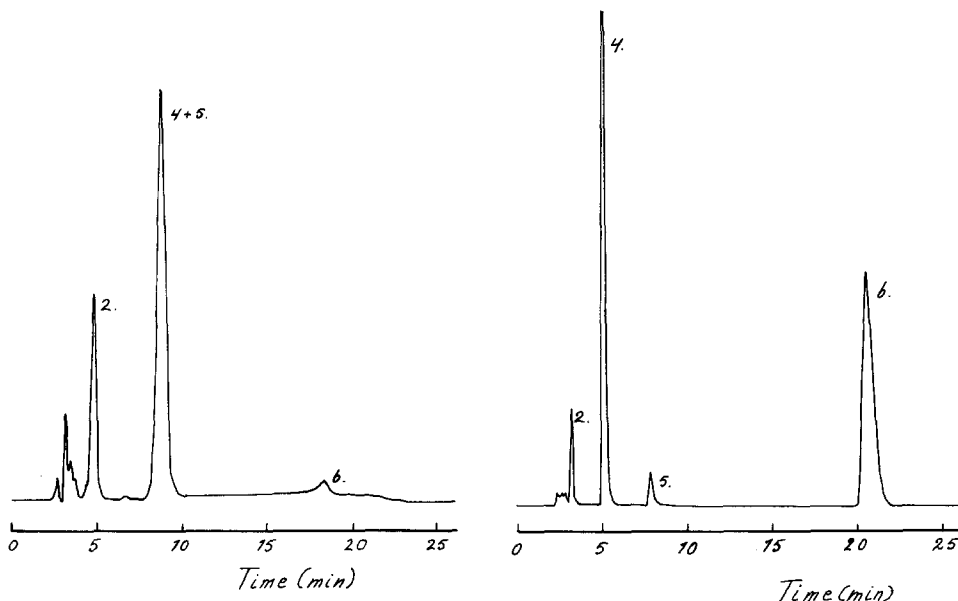


Fig. 4. Chromatogram of a sample of a reaction mixture of phenol and a three-fold excess of glyoxylic acid obtained with the column-detection combination SCX,UV. Analytical conditions and peak numbering as in Fig. 3.

Fig. 5. Chromatogram of the same sample as used in Fig. 2 obtained with the column-detection combination ODS,UV. Analytical conditions as given in the Experimental section. Flow-rate of the eluent: 0.8 ml/min. Peak numbering as in Fig. 2.

3, it appears that the disubstituted phenol (ophma) is eluted immediately after glyoxylic acid. An additional indication for the assignment of ophma is given by Fig. 4. This figure shows a chromatogram of a sample taken from a reaction mixture with about a three-fold excess of glyoxylic acid using the combination SCX,UV. Comparing Figs. 3 and 4, it appears that the elution peak assigned to ophma is considerably higher for the sample from the reaction with excess of glyoxylic acid than for the sample from the reaction with equimolar amounts of phenol and glyoxylic acid. This is in accord with the scheme in Fig. 1, from which it can be derived that the amount of ophma increases with increasing excess of glyoxylic acid.

Fig. 5 shows a chromatogram obtained with the combination ODS,UV. For this chromatogram the sample was the same as that used as for the chromatograms shown in Figs. 2 and 3. From Figs. 3 and 5 it can be concluded that the selectivity of the aromatic compounds is much better for the ODS column than for the SCX column. Chromatograms obtained with the combination ODS,RI show that glyoxylic, glycolic and oxalic acid are eluted in the injection peak. The elution volumes of the aromatic compounds in the reaction mixture increase in the order: ophma < phma < ohma < phenol.

For each of the aromatic compounds present in the reaction sample the capacity factor,  $k'$ , was calculated as described by Simpson<sup>7</sup>. For both columns, Fig. 6 shows plots of the capacity factors as a function of the number of  $\alpha$ -hydroxyacetic acid groups present in the aromatic ring. It is seen that  $k'$  decreases in the order

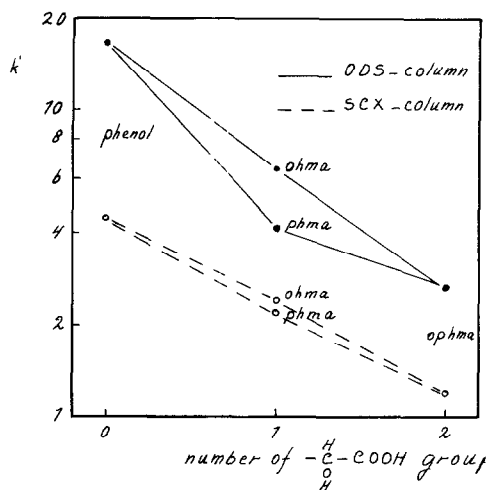


Fig. 6. Capacity factors of the aromatic compounds as a function of the  $\alpha$ -hydroxyacetic acid groups bound to the aromatic ring.

phenol > ohma > phma > ophma, for both columns used. The similarity of the behaviour of  $k'$  for the SCX and the ODS column suggests an identical separation mechanism for these columns.

Fig. 6 clearly demonstrates that the retention in the ODS column originates from hydrophobic interactions between the aromatic compounds and the non-polar stationary phase. The introduction of only one polar  $\alpha$ -hydroxyacetic acid group to phenol results in a considerable decrease in the magnitude of the hydrophobic interactions. This is expressed as a strongly decreased retention time on going from phenol to ohma or phma. The different retention behaviours of ohma and phma can be explained by realizing that the hydrophobic interactions between the unoccupied part of the aromatic ring and the stationary phase are probably stronger for ohma than for phma. A retention mechanism for the SCX column based on the hydrophobic interactions between the resin matrix and the aromatic ring is in accord with the conclusions of Jandera *et al.*<sup>3</sup> and Jahangir and Samuelson<sup>4</sup>.

The quantification of the reaction mixture was carried out with the combinations SCX,RI and ODS,RI. The latter combination is necessary for the quantification of the aromatic compounds. The concentrations of ohma were calculated with the assumption that the molar responses of phma and ohma are equal for refractive index detection. The molar response of ophma was calculated by assuming a linear relationship between the molar response and the number of  $\alpha$ -hydroxyacetic acid groups present. With these assumptions the mass balance of the reaction mixtures was complete for phenol conversions up to 100%.

At higher conversions the quantification of glyoxylic acid is hampered by the partial overlap of its elution peak with that of the disubstituted product. At rather low conversions (< 50%), the determination of the concentration of glyoxylic acid is possible with an acceptable accuracy, the concentration of the disubstituted product in the samples being relatively low.

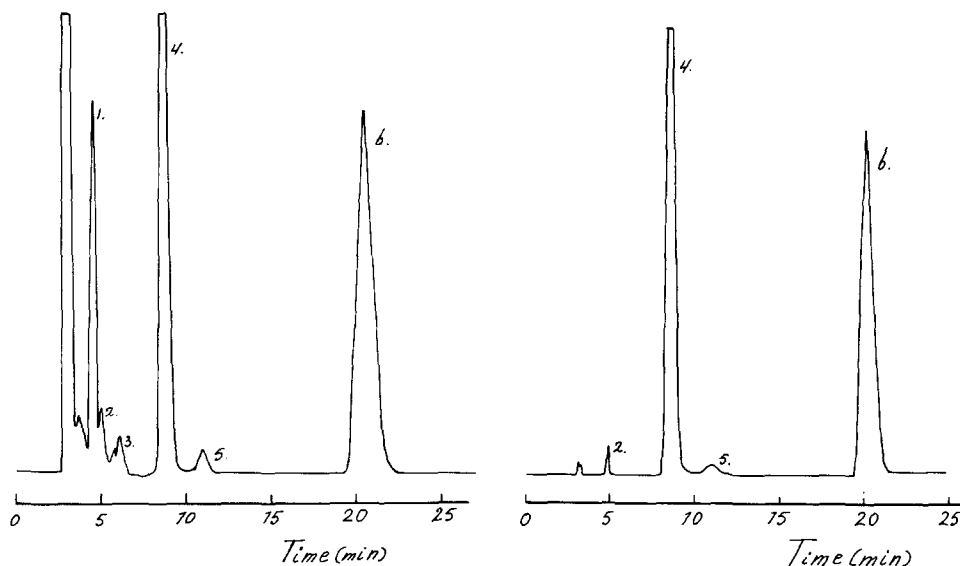


Fig. 7. Chromatogram of a sample from a reaction mixture of guaiacol and glyoxylic acid obtained with the column-detection combination SCX,RI. Analytical conditions as in Fig. 2. Peaks: 1 = glyoxylic acid; 2 = disubstituted product; 3 = glycolic acid; 4 = *para*-substituted product; 5 = *ortho*-isomer of compound 4; 6 = guaiacol.

Fig. 8. Chromatogram of the same sample as used for Fig. 7 obtained with the column-detection combination SCX,UV. Analytical conditions and peak numbering as in Fig. 7.

#### Reactions of quaiacol and glyoxylic acid

The chromatograms obtained with the combinations SCX,RI and SCX,UV are shown in the Figs. 7 and 8, respectively. The assignment of the peaks in the chromatograms is based on the same arguments as those used for the reactions of phenol and glyoxylic acid. It is striking that the product *D,L*- $\alpha$ , 4-dihydroxy-3-methoxybenzeneacetic acid and its isomer *D,L*- $\alpha$ , 2-dihydroxy-3-methoxybenzeneacetic acid are fully separated on the SCX column. The disubstituted product  $\alpha,\alpha'$ , 4-trihydroxy-5-methoxy-1,3-benzenediacetic acid is almost completely separated from glyoxylic acid. It appears that the retention volumes of guaiacol and *D,L*- $\alpha$ , 4-dihydroxy-3-methoxybenzeneacetic acid are much higher than the corresponding values for phenol and phma under the same chromatographic conditions. These differences can be explained by the presence of a methoxy group in guaiacol and its  $\alpha$ -hydroxyacetic acid derivatives. This methoxy group is probably responsible for an additional hydrophobic interaction with the non-polar parts of the resin matrix. This explanation is supported by the results of Molnar and Horváth<sup>8</sup>, who obtained similar results for *D,L*- $\alpha$ , 4-dihydroxy-3-methoxybenzeneacetic acid and phma on a RP-18 stationary phase.

For the quantification of the aromatic compounds in the reaction mixture the combination ODS,RI was used. The aromatic components in the reaction mixture are also completely separated. The same assumptions were made as for the system phenol-glyoxylic acid. Since *D,L*- $\alpha$ , 4-dihydroxy-3-methoxybenzeneacetic acid was not



available in an acceptable purity, the difference between the molar responses of this product and guaiacol is assumed to be equal to the difference in the molar responses of phma and phenol. With these assumptions a complete mass balance for the aromatic compounds was obtained for guaiacol conversions up to 100%.

#### ACKNOWLEDGEMENT

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