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STATIONARY PHASE CHARACTERIZATION IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

A TEST FOR TRACE METAL ACTIVITY IN OCTADECYL BONDED SILICA GEL

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SUMMARY

In this paper it is shown that metal traces in high-performance liquid chromatographic bonded-phase silica gel interfere with some separations. A test for such activity, based on the chromatography of beer bitter acids, is described. When octadecyl silica gel is boiled in concentrated hydrochloric acid–methanol (40:60), part of the phase is dissolved; however, the metal traces are selectively removed and the quality of the phase improved.

INTRODUCTION

The highest plate numbers for reversed-phase high-performance liquid chromatography (HPLC) columns are obtained with polycyclic aromatic hydrocarbons; in general, the plate number drastically decreases with other homologous series containing heteroatoms. This is even the case with the simple compounds of Table I.

The plate numbers in Table I were calculated from the peak width at half the peak height. The k values were calculated from the T_0 value of the peak produced by 10 μl of a 0.01 % methanol–water (70:30) solution of KBr measured at 200 nm, as described by Berendsen *et al.*[1]. Column 1 is filled with commercial octadecyl silica gel (of good quality when compared to other commercial reversed-phase packings) and column 2 is filled with the same material, but purified by twice boiling with 6 N HCl followed each time by extensive washing. Boiling with hydrochloric acid removes most of the metals[2] and column 2 clearly gives better results for cinnamic acid. With amines, *e.g.*, benzylamine, this phenomenon is even more spectacular. The amine must be chromatographed separately as it would interfere with the peaks of the polarity test mixture of Table I. With benzylamine under the conditions of Table I, column 1 gives only a rise in the baseline, spread out over a large part of the chromatogram. Column 2 gives a peak, although only with 130 plates. That benzyl alcohol and benzaldehyde give lower plate numbers is partly due to their low k values. For cinnamic acid it is clear that another factor is involved, and we attribute the low

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TABLE I

PLATE NUMBERS OF HPLC COLUMNS WITH COMPOUNDS OF A POLARITY TEST MIXTURE

Column (25 × 0.46 cm) of 10-μm octadecyl silica gel eluted with methanol–water (60:40) at 120 ml/min. k = Capacity factor.

Compound		*Column 1*		*Column 2*	
		k	*Plate No.*	*k*	*Plate No.*
1	Benzyl alcohol	1.6	3400	1.5	3170
2	Benzaldehyde	2.4	2940	2.2	2860
3	Acetophenone	2.8	4450	2.6	4210
4	Cinnamic acid	3.7	970	3.0	2280
5	Anisole	5.4	5570	4.8	5650
6	Benzyl chloride	8.3	5830	7.0	5590
7	Toluene	10.9	6320	9.3	6270
8	1-Nitronaphthalene	12.0	5510	10.4	5400
9	Naphthalene	18.3	6230	15.3	6280

plate number on column 1 to trace metal ions in the silica gel matrix. This seems particularly harmful for acids and bases, but then many of the mixtures analysed in HPLC are or contain bases or acids. Buffering the solvents or adding anti-complexing compounds is not always effective, as shown for strongly chelating hop bitter acids[3].

Undoubtedly, the trace metal impurities in most commercial silica gel phases are harmful to many separations. It is therefore of interest to devise a method for evaluating this property of silica gel HPLC phases. As reversed-phase HPLC has now become the most important form of liquid chromatography, we have developed such a method for this stationary phase. It uses the bitter acids which can easily be extracted from beer and which are thus widely available.

RESULTS AND DISCUSSION

A series of hop (derived) bitter acids is shown in Fig. 1. Complex formation or interaction with trace amounts of metals is based on a planar *cis* enolized β-carbonyl system capable of strong chelation. In compound III, deacylated antiisohumulone, this feature is lacking, and the HPLC peak of III is not deformed even on material which otherwise gives poor results on account of traces of metals. All the other compounds have the chelating structure but the strength of the acidic vinyl function is also important. Complexation in acidic media decreases in the following series: humulinic acids ($pK \approx 3$) > iso-α-acids ($pK \approx 3.2$) > α-acids ($pK \approx 5.5$) > β-acids ($pK \approx 6.0$). This is reflected in the chromatograms in Figs. 2 and 3. These were recorded under the same conditions on a thoroughly demineralized commercial HPLC octadecyl silica gel (Fig. 2) and on insufficiently deionized material (Fig. 3). Peak 1, which is deacylated antiisohumulone, behaves normally on both phases as expected. The humulinic acids exhibit pronounced tailing in Fig. 3 and the iso-α-acids show a decided loss of resolution. These phenomena are due to the activity of traces of metals and thus can be exploited to develop an activity test for alkyl bonded-phase silica gels. Without phosphoric acid as anti-complexing agent, none of the many commercial octadecyl bonded silica gels we tested gave satisfactory results. In this context, when

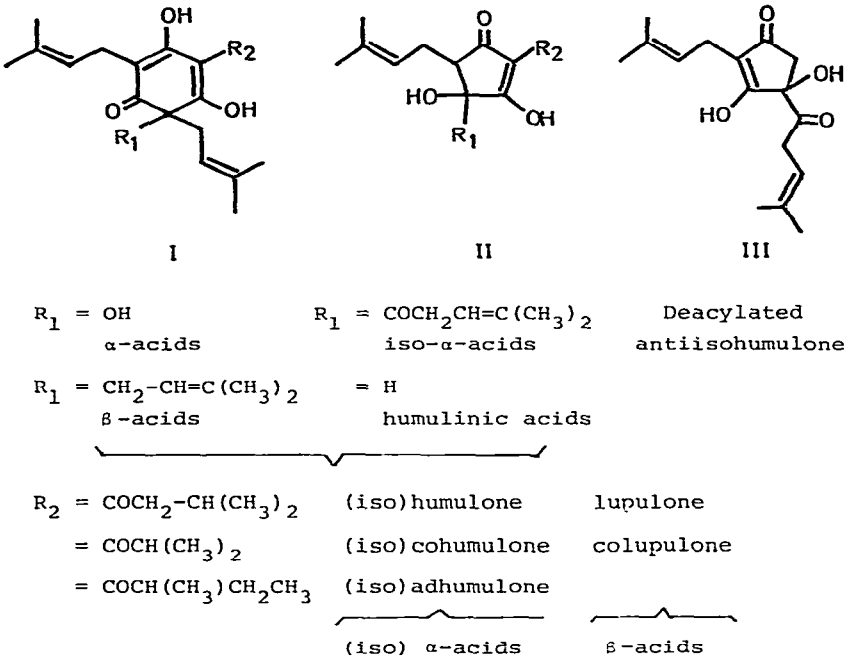


Fig. 1. Formulae of hop bitter acids and derived products.

possible and compatible with the sample, we always add some phosphoric acid to eluting solvents in bonded-phase HPLC.

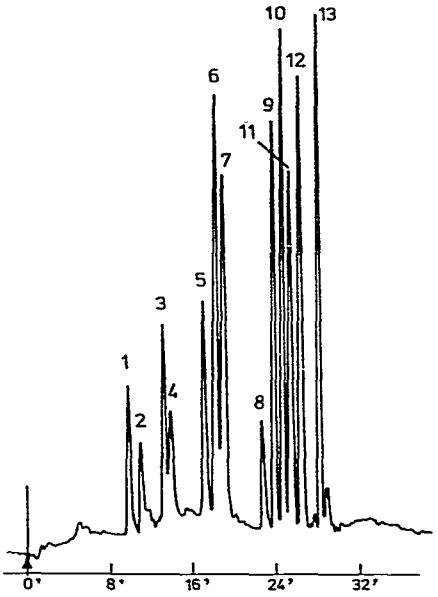
Activity test

It is difficult to develop a quantitative test for traces of metals. Owing to (1) the great difference in activity and even in chromatographic behaviour of commercial bonded silica gel phases, and (2) the unavailability of a 100% pure hop bitter acid or even of a mixture with exactly known composition. Still, a very sensitive qualitative test can be based on the chromatographic behaviour of beer iso-α-acids. All beers contain about 15–35 ppm iso-α-acids (lager or pale yellow beer), sometimes up to 80 ppm (some top fermentation dark coloured beers). European lagers produced on a large scale generally contain 25 ppm ± 10% if iso-α-acids; American lagers are less bitter. The iso-α-acids can easily and selectively be extracted with isooctane.

Procedure

A 20-ml volume of beer is acidified with 2 ml of 3 *N* HCl (or an equivalent amount) and hand extracted in a separating funnel with 50 ml isooctane. Although some emulsion may form, 25 ml of the isooctane can be removed and evaporated on a vacuum rotatory evaporator (rotavapor). The residue is dissolved in 1 ml methanol.

Phenanthrene is used as standard and a solution in methanol (5 mg per 100 ml) is prepared. It is not known in advance whether the standard will be coeluted with one of the iso-α-acids peaks, this depending on the particular bonded phase material. Therefore the standard can be chromatographed separately.



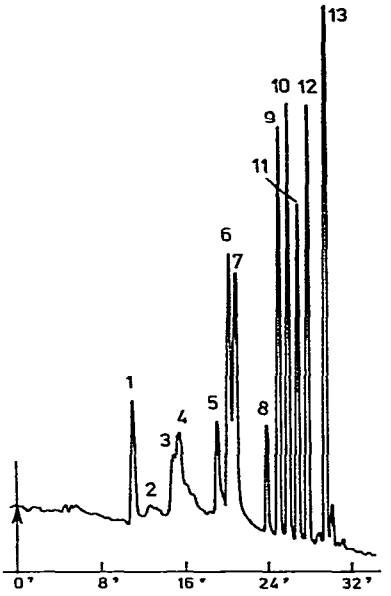


Fig. 2. HPLC on Varian 5020 LC. Column (25 m × 0.46 cm) of 10-μm RSiL-C_{18}-HL-D which had been boiled three times with 6 *N* HCl. Detection at 280 nm (Vari-chrom). Solvent: methanol–water–phosphoric acid (85%) with gradient from 50:50:0.5 to 100:0:0.5 in 30 min at 2 ml/min. Sample loop injection (Valco): 10 μl, 7000 p.s.i. Pressure from 300 to 150 kg/cm². Sample mixture: 1 = deacylated antiisohumulone; 2 = *trans*-cohumulinic acid; 3 = *trans*-humulinic acid; 4 = *trans*-adhumulinic acid; 5 = *trans-allo*-isohumulone; 6 = *trans*-isohumulone; 7 = *trans*-isoadhumulone; 8 = cohumulone; 9 = humulone; 10 = exo-TCOC (tricyclooxycolupulone); 11 = colupulone; 12 = lupulone; 13 = hexahydrocolupulone. Compounds 5, 10 and 13 are not mentioned in Fig. 1; 5 is like 6 but with a shift of a side chain double bond; 10 and 13 are colupulone derivatives.

Fig. 3. Chromatogram obtained under the same conditions as in Fig. 2, except that the RSiL-C_{18}-HL-D had not been boiled with hydrochloric acid.

Chromatography is carried out with sample loop injection of 10 μl and with isocratic elution [methanol–water–85% phosphoric acid (75:25:0.5)] at 2 ml/min and with UV detection at 280 nm. The beer extract sample and phenanthrene are chromatographed one after the other. If coelution does not occur, the phenanthrene can of course be added to the sample as an internal standard. Chromatograms on different phases (Fig. 4) show how difficult it is to sufficiently demineralize bonded phase silica gel. On thoroughly demineralized bonded phase silica gel, good chromatograms are obtained even without phosphoric acid as complexing agent. Another acid, *e.g.*, formic acid, has then to be used to bring the pH down to ≈ 2.5. Higher concentrations of phosphoric acid than the 0.5% advocated do not improve peak shape; they increase the retention time of the iso-α-acids. Ethylenediaminetetraacetate (EDTA) has a positive effect, although it is only sparingly soluble in acidic media. Based on these results, the following activity test can then be performed.

The beer extract sample is chromatographed isocratically with the following solvent mixtures:

(1) methanol–water–formic acid (75:25:0.5)
(2) methanol–water–phosphoric acid (75:25:0.5)

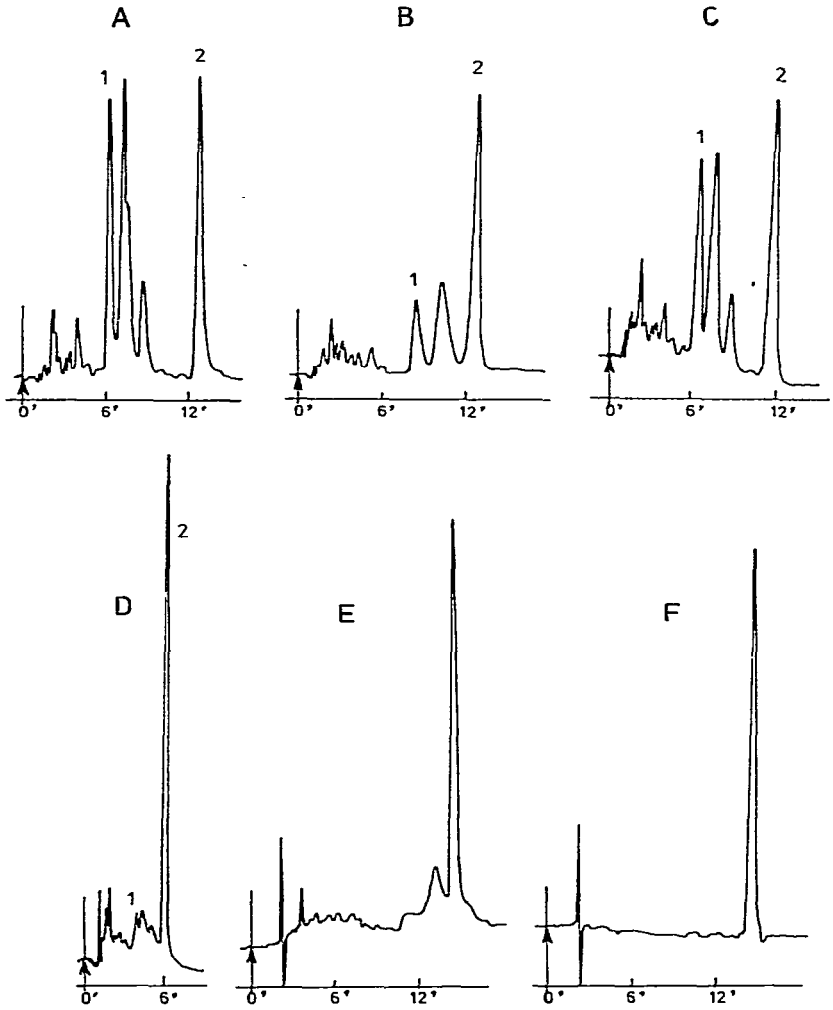


Fig. 4. Comparative chromatograms (HPLC) of beer extract and (internal) standard. Sample loop injection (Valco): 10 μl, 7000 p.s.i. Isocratic elution with methanol–water–phosphoric acid (85%) (75:25:0.5) at 2 ml/min. UV detection at 280 nm. A. Thoroughly demineralized commercial octadecyl bonded silica gel; B, the same stationary phase, untreated with HCl; C, the same material as A and B boiled twice with 1 *N* HCl; D, commercial spherical octadecyl silica gel; E, another commercial spherical octadecyl silica gel; F, the reference phenanthrene. Peaks: 1 = isocohumulone; 2 = phenanthrene.

(3) as 2 but saturated with EDTA (solubility of EDTA is only in the ppm range — saturation is achieved by adding 20 ppm, shaking and standing overnight).

The height of the isocohumulone peak (first of the three iso-α-acids) is then divided by the height of the phenanthrene peak. This leads to a height ratio (HR) value (Table II).

In this way we have analysed many commercial materials, with irregular and spherical particle shapes. None merits the lable "excellent". Most are bad to very bad.

TABLE II

HEIGHT RATIO (HR) VALUES WITH DIFFERENT SOLVENTS AND QUALITIES OF BONDED-PHASE SILICA GEL

Solvent	*HR values*			
1	>0.5	≈0	≈0	≈0
2	>0.5	>0.5	0–0.5	>0
3	>0.5	>0.5	>0.5	0–0.5
Quality	Excellent	Reasonable	Bad	Very bad

For this reason we do not mention explicit brand names. By boiling some bonded phases twice for 4 h with 1 *N* HCl, followed by extensive washing, they can be brought to the status "reasonable". The label excellent is only achieved by boiling at least three times with 6 *N* HCl.

Boiling of reversed-phase silica gel with methanolic hydrochloric acid was studied in more detail as follows. Twenty grams of RSIL-C_{18}-D 10-μm reversed-phase silica gel dried at 70°C were boiled for 2 h in 60 ml methanol and 40 ml concentrated hydrochloric acid. After thorough washing with water and drying at 70°C, a weight loss of 5% was recorded. The amount of bonded phase as estimated by thermogravimetric analysis (TGA) before and after the treatment had also dropped by about 5% (from ≈18 to 17%). Foaming of the boiling suspension also indicates loss of octadecyl groups.

On repeating the procedure with the obtained material a new loss of 3% was measured. According to a silanol activity test devised recently[4], the acid-treated material still behaves as a deactivated phase. The drop in k value observed between columns 1 and 2 in Table I shows that the acid treatment removes some octadecyl groups. This is not unexpected for a surface reaction on surface bonded material. It is surprising that not more of the 5% loss on boiling with acid is due to loss of octadecyl groups. The above observations seem to indicate that the acid treatment cleaves the Si–O bonds but not the Si–C bonds. This is also the case in alkaline hydrolysis[5].

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