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Note

Use of a stop-flow technique to study on-column decomposition in supercritical fluid chromatography

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The on-column decomposition of analytes during chromatography can lead to reductions in peak area, shifts of baseline and in extreme cases complete loss of the analyte peak and appearance of new peaks. Whilst relatively common in gas chromatography (GC)[1–3], the phenomenon seldom occurs in liquid chromatography (LC) and a similar situation might be expected to apply to packed-column supercritical fluid chromatography (SFC).

During the course of a study of drug metabolites by SFC inconsistent quantitative results were observed for N-oxides[4]. Since for the same calibration solution peak areas appeared to vary with column residence time on-column decomposition was suspected. In order to test this proposition a systematic study has been performed using test solutes substances, that also are amenable to gas and liquid chromatography, for which the decomposition chemistry is well understood.

The results of this work, which clearly demonstrate that on-column decomposition can lead to errors in packed-column SFC now are presented.

EXPERIMENTAL

Apparatus

SFC was performed on 100 mm × 4.6 mm I.D. stainless-steel columns packed with 5-μm Spherisorb silica, 5-μm aminopropyl silica or 7-μm carbon black (Shandon, Runcorn, U.K.). Columns were heated in a Model TC 1900 oven (ICI Scientific Instruments, Dingley, Australia) at temperatures up to 91°C. The mobile phase consisting of mixtures of carbon dioxide (British Oxygen Gases, London, U.K.) and methanol was pumped through the system by Model 302 and 303 piston pumps (Gilson, Middleton, WI, U.S.A.) controlled by means of an Apple II GS microcomputer. The pump head was cooled to −15°C by means of a RTE-4 refrigerated bath circulator (Jencons, Leighton Buzzard, U.K.) to facilitate the filling of the pump with liquid mobile phase. Samples were introduced by means of a Rheodyne Model 7125 injector fitted with a 20-μl sample loop. The column effluent was monitored by a Model 757 variable wavelength UV detector (Kratos Analytical, Ramsey, NJ,

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U.S.A.) set at 254 nm. A Tescom back-pressure regulator (Tescom Instruments, Elk River, MI, U.S.A.) was used to maintain supercritical conditions.

The high-performance LC (HPLC) system consisted of Model PU 4015 piston pump (Philips Analytical, Cambridge, U.K.), a Rheodyne Model 7125 injector fitted with a 10-μl injector loop and a Model PU 4025 UV detector (Philips Analytical) operated at 254 nm. Chromatography was performed on 250 mm × 4.6 mm I.D. stainless-steel columns packed with 10-μm Partisil ODS-1 or 5-μm Spherisorb silica (Phase Separations, Queensferry, U.K.). The columns were thermostated by means of a Model TC 1900 oven (ICI Scientific Instruments).

The GC experiments were carried out on a Model 204 gas chromatograph (Philips Analytical) equipped for packed and capillary column operation with on-column injection and flame ionization detection. Chromatography was performed on 2 m × 4 mm I.D. glass columns packed with 10% (w/w) OV-1 or PEG 20M on 80–100 mesh Chromosorb P and a 10 m × 0.32 mm I.D. fused-silica capillary coated with PEG 20M, film thickness 0.5 μm (Thames Chromatography, Maidenhead, U.K.). Nitrogen or helium was used as carrier gas with a Model 151/3G toggle valve (Hoke International, Harrow, U.K.) in the gas line to enable stop-flow operation.

Materials

HPLC-grade methanol and acetonitrile were obtained from BDH (Poole, U.K.) whilst 2-phenylpropan-2-ol, 1-phenylpropan-2-ol, 2-phenylpropan-1-ol and propiophenone were obtained from Aldrich (Gillingham, U.K.).

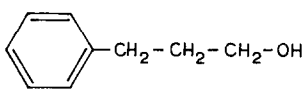
RESULTS AND DISCUSSION

There is increasing evidence to suggest that SFC is appropriate for the assay of thermally liable compounds[5–7]. Normally such compounds are polar in nature and require the use of mobile phase with high percentages of polar modifiers, such as methanol, which in turn necessitate operation at elevated temperatures to maintain supercritical conditions. For instance, Cheuh and Prausnitz[8] have shown that for a carbon dioxide–methanol (80:20, v/v) mixture column temperatures in excess of 75°C are necessary. At such temperatures on-column degradation of thermally liable compounds is a possibility, especially with silica-based stationary phases, where acid-catalysed elimination reactions can occur. In order to test this thesis a series of phenylalkanols with primary, secondary and tertiary hydroxyl groups have been examined by stop-flow SFC and for comparison by GC and LC.

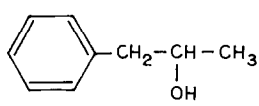
Rapid on-column reactions generally are associated with the appearance of additional peaks due to the fragmentation of molecules. On the other hand relatively slow on-column reactions lead to apparently normal chromatograms but with reduced peak areas which lead to unsuspected quantitative errors. Two techniques may be used to detect on-column reactions which are not manifested by the appearance of additional peaks or baseline disturbance. The first involves the measurement of peak areas for a labile test substance relative to those of a stable internal standard for a range of column temperatures. Here reductions of retention and associated peak areas at the higher temperatures can lead to uncertainties, also comparisons are complicated by differences in solute residence times. These limitations can be overcome by the second method, stop-flow chromatography, which involves interuption of the elution process

by stopping the mobile phase flow when the solutes are midway down the column. The analyte and internal standard thus trapped on the column continue to undergo dynamic partition and or adsorption equilibria and any associated chemical reactions. Subsequent elution of the solutes by resumption of the mobile phase flow yields a chromatogram from which peak area measurements may be made to indicate the extent of analyte decomposition. The latter approach was preferred in the present work.

As test compounds were chosen a range of phenyl propanols with increasing ease of dehydration due to the presence of primary (I), secondary (II) or tertiary (III) hydroxyl groups.



I



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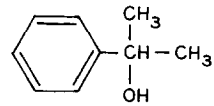
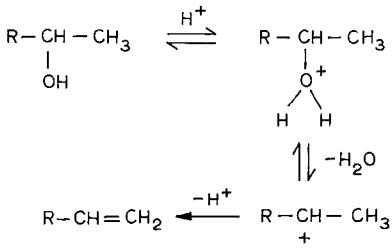


III

In the presence of acidic catalysts the alcohols undergo an E1 reaction[9] following initial protonation to yield conjugated propenylbenzenes via a carbocation:



Silica-based stationary phases, which are commonly used in chromatography, have acidic surfaces owing to the presence of silanol groups which could act as a source of protons[10] for the initial step of the decomposition reaction.

In a preliminary experiment all three alcohols were studied by stop-flow SFC using a silica column at 91°C with an input pressure of 141 bar. For each measurement the mobile phase flow was stopped once the sample was judged to be half-way down the column. Peak areas of test alcohol and propiophenone (internal standard) were measured on the resumption of flow. Taking a normal chromatogram as a reference point the peak area ratios were used to calculate the relative reaction rates shown in Fig. 1. As expected the tertiary alcohol displayed the greater rate of dehydration. Furthermore the results suggest that significant quantitative errors could arise except where high mobile phase flow-rates are employed. On-column decomposition of the primary and secondary alcohols in contrast was insignificant.

Bonded phases with reduced silanol contents would be expected to be less catalytic and this is borne out in practice as illustrated by data obtained using an

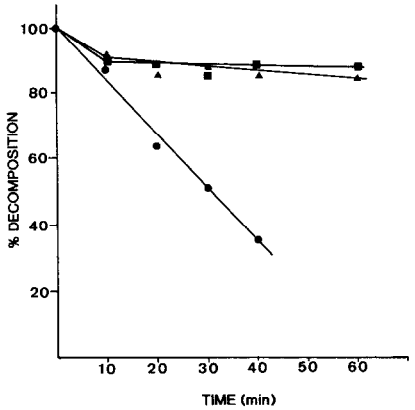


Fig. 1. On-column decomposition of phenylpropanols during stop-flow SFC on a 5-μm Spherisorb silica column at 91°C with carbon dioxide–methanol (99:1, v/v) mobile phase and UV detection at 245 nm. Compounds: ■ = I; ▲ = II; ● = III.

aminopropyl column, see Fig. 2. Even better results were obtained using a graphitized carbon black column where no discernible decomposition occurred. However, retentions on this phase were significantly greater than on the silica-based phases, owing to its greater affinity towards aromatic compounds.

No significant on-column decompositon of 2-phenylpropan-2-ol was found to occur during reversed-phase LC under normal conditions. However, in the presence of acidic eluents and elevated column temperatures moderately rapid decomposition

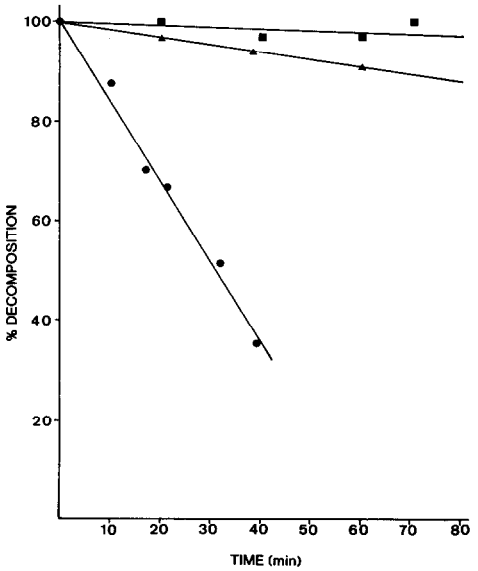


Fig. 2. Effect of stationary phase upon the on-column decomposition of 2-phenylpropan-2-ol during stop-flow SFC at 91°C. Stationary phases: ■ = graphitized carbon black; ▲ = aminopropyl silica; ● = silica gel.

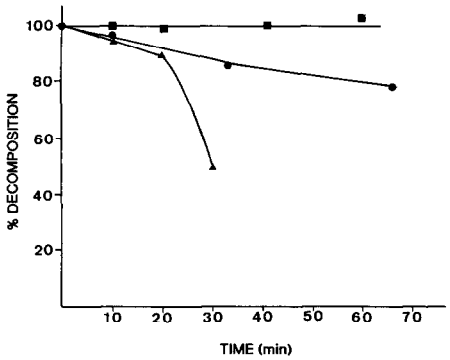


Fig. 3. On-column decomposition of 2-phenylpropan-2-ol during stop-flow normal- and reversed-phase liquid chromatography. ▲ = 5-μm Spherisorb silica at room temperature with *n*-heptane–isopropanol (97.5:2.5, v/v) as eluent; ● = 10-μm Partisil ODS at 81°C with acetonitrile–0.1 *M* phosphoric acid (55:45, v/v) as eluent; ■ = 10-μm Partisil ODS at room temperature with acetonitrile–water (55:45, v/v) as eluent. Propiophenone used as internal standard.

occurred under stop-flow conditions, as shown in Fig. 3. Under normal-phase conditions with silica gel as stationary phase and *n*-heptane–isopropanol as eluent rapid analyte decomposition occurred even at room temperature. It is interesting to note that 2.5% of isopropanol in the mobile phase was apparently insufficient to prevent protonation of the analyte molecules leading to their decomposition.

Consistent with previous observations[11] on-column reactions were found to occur during stop-flow GC on both OV-1 and PEG 20M packed columns. On the other hand, no decomposition was apparent with the PEG 20M fused-silica capillary column as might have been expected. However, the results in the latter case were less conclusive owing to the extensive zone dispersion which occurred whilst the carrier gas flow was suspended. An observation that is consistent with the differences between analyte gas phase diffusion in open-tubular and packed chromatographic columns[12].

The results of this work demonstrate that caution should be exercised when analysing labile substances, particularly those prone to acid catalysed elimination reactions, on silica-based columns, not only in GC but also LC and SFC.

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