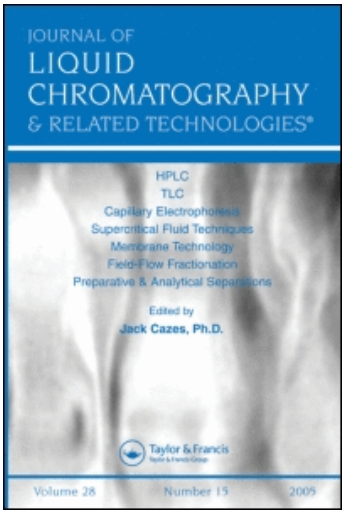

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Solid Phase Derivatization Reactions in HPLC Polymeric Reductions for Carbonyl Compounds

I. S. Krull[a]; K-H. Xie[a]; S. Colgan[a]; U. Neue[b]; T. Izod[b]; R. King[b]; B. Bidlingmeyer[b]
[a] Institute of Chemical Analysis and Department of Chemistry, Northeastern University, Massachusetts, USA [b] Research and Development Departments Waters Associates, Inc., Milford, Massachusetts, USA

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SOLID PHASE DERIVATIZATION REACTIONS IN HPLC
POLYMERIC REDUCTIONS FOR CARBONYL COMPOUNDS

I.S. Krull*, K-H. Xie, and S. Colgan
Institute of Chemical Analysis and
Department of Chemistry
Northeastern University
360 Huntington Avenue
Boston, Massachusetts 02115 USA

and

U. Neue*, T. Izod, R. King, and B. Bidlingmeyer
Research and Development Departments
Waters Associates, Inc.
34 Maple Street
Milford, Massachusetts 01757 USA

ABSTRACT

Polymeric reducing agents, such as polymeric borohydride resins, can be successfully utilized in both the pre- and post-column modes, on-line, at ambient temperatures, in real-time, for the selective chemical reduction of a large number of aldehydes. Other classes of carbonyl derivatives, such as ketones, esters, amides, etc., are totally unreactive under these same polymeric reduction conditions. This particular reducing agent is fully compatible with conventional reversed phase HPLC columns and mobile phases, such as methanol/water, ethanol/water, and acetonitrile/water, in varying proportions. The approach to selective detection in HPLC described here utilizes conventional UV-VIS detection following pre- or post-column reductions that occur on-line. Alternative detection methods could just as readily be utilized. Overall reactions are rapid, quantitative, reproducible, and highly selective. In certain cases, it is possible to realize improved detection limits as a result of aldehyde reductions. Difference chromatography or changes in detector responses for unresolved reactant and product can be used to denote reactions that have occurred on the original analyte(s) of interest. Polymeric reduction columns can be slurry packed at fairly low pressures, and they are stable at elevated HPLC back pressures for prolonged periods of time and use. The use of polymeric reagents in HPLC is a totally general approach to improved specificity, selectivity, and detection limits in all types of organic and inorganic analyses.

*Authors to whom correspondence and reprint requests should be addressed.

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INTRODUCTION (24)

High performance liquid chromatography (HPLC) has rapidly become one of the most powerful, useful, and popular methods of organic or inorganic analysis. Although a wide variety of packing materials and separation approaches have been developed over the years, there has been a serious lack of advancement made in the important area of detectors for HPLC (1-5). Still today, the most widely used HPLC detectors are limited to the following: 1) ultraviolet/visible (UV/VIS); 2) fluorescence (FL); 3) refractive index (RI); and 4) electrochemical (EC). By far, the greatest number of analyses are still done utilizing UV/VIS, although EC is rapidly becoming more and more widely employed for those compounds that are or can become electrochemically active. In order to improve the number of applications of UV/VIS or EC, as well as to improve overall specificity and detection limits, more and more analysts have turned to a wide variety of readily available derivatization techniques (6-10). Indeed, of late, various HPLC instrument manufacturers have developed and commercialized certain automated, on-line, post-column or pre-column (off-line) derivatization equipment for performing many routine homogeneous derivatizations automatically. Except for a very limited number of reported studies, virtually all of the currently used derivatization schemes for HPLC utilize homogeneous reaction conditions (10). This requires the mixing, usually after the analytical column, on-line, of the individual eluting analytes with the derivatization reagent solution(s), followed by a reaction chamber, often at elevated temperatures, followed by the general detector (UV/VIS, etc.). This homogeneous derivatization approach has been very widely adopted and adapted for a large number of HPLC applications, despite the fact that it has serious disadvantages and drawbacks. These have been summarized in Table 1.

Heterogeneous derivatization reactions, wherein the reagent is present as a solid, supported on an inert media such as silica gel or alumina, or covalently or ionically attached to a polymeric support, have been used in HPLC only a few times. This area has recently been reviewed by Krull and Lankmayr (10). Although supported reagents, such as sodium borohydride ($NaBH_4$) on silica gel or alumina, as well as polymeric reagents, such as polymeric borohydride, have been described for many years in the synthetic organic literature, they have rarely been described in any HPLC related interfacing or applications (11-20). We propose to term this entire approach for HPLC derivatization, on-line or off-line, as solid phase reactors (SPRs) or solid phase derivatization reactions. It should become immediately clear that we have chosen to study but one particular type of solid phase reactor, the polymeric borohydride reduction system, but that there remain a very large

TABLE 1

DISADVANTAGES OF HOMOGENEOUS DERIVATIZATIONS

1. Requires the use of additional instrumentation, HPLC pumps, mixing chamber, reaction chamber, heating arrangements, connectors, plumbing lines.
2. Introduces additional extra-column dead volume that adversely affects overall HPLC performance.
3. Increases overall time for each analysis.
4. Increases overall costs per analysis.
5. Possible source of contamination via reagent solutions, solvents, extra equipment and instrumentation, etc.
6. Underivatized, excess derivatizing reagents are always present, thus raising background noise levels and increasing (worsening) detection limits.
7. Cannot routinely be used as an on-line, pre-column approach. Restricted to post-column derivatization in on-line mode.
8. Often requires additional sample handling and manipulation, especially in the off-line approach.

number and types of solid phase reactors that could just as easily be introduced and applied in various HPLC applications and settings.

Solid phase derivatization reactions provide a number of important and valuable advantages when compared with the more conventional homogeneous type derivatizations. These have been summarized in Table 2. It becomes clear that solid phase derivatization approaches offer significant advantages over most of the previously used homogeneous solution type derivatizations for HPLC. We describe here what would appear to be one of the first true uses of any supported or polymeric reagents for trace organic/inorganic analyses and speciation *via* conventional HPLC-UV methods. Although this study has been devoted to a polymeric type reagent, we already have sufficient data and information in-hand to indicate that silica gel or alumina supported reagents are just as useful and applicable to HPLC-detector interfacing. These results will be reported at a later date. We report the evaluation of a polymeric borohydride resin incorporating sodium borohydride, for performing on-line, real-time, chemical reductions of various aldehydes using reversed phase separation conditions and mobile phases. At the same time, we have partially demonstrated the potential usefulness of this approach in the analysis of cinnamaldehyde in two different consumer product samples, a formulated spice product (cinnamon) and a mouthwash solution.

TABLE 2

ADVANTAGES OF SOLID PHASE DERIVATIZATION REACTIONS

1. Does not require additional instrumentation, hardware, mixing chambers, or reaction chambers. Only requires solid phase reactor, dummy reactor, and fittings.
2. Does not introduce any additional extra-column dead volume other than that normally introduced with any conventional guard column.
3. Usually will not increase overall time for each analysis.
4. Slight increase in overall cost per analysis.
5. Does not introduce contamination from solid phase reactor.
6. There is no derivatizing reagent present in mobile phase leading to no increased background noise level. Detection limits are not adversely affected, but are generally improved.
7. Can be used in either the pre- or post-column modes, using differences in overall chromatography (pre-) or differences in detector responses (post-).
8. Does not require any additional sample handling or manipulations, only requires direct injection onto HPLC with and without solid phase reactor on-line.
9. Can be fully compatible with wide variety of mobile phases, for both normal phase and reversed phase HPLC.
10. Reactions can often occur in real-time, with no increased analyte hold-up prior to the column or detector.
11. Since reactions occur on a solid support, most will occur at or slightly above room temperature.
12. Derivatization reactions are more selective and specific than for same reagent(s) in solution.

EXPERIMENTAL

Reagents

The initial sample of the polymeric borohydride resin, borane poly-(2-vinylpyridine) complex, was obtained from Alfa Products, Ventron Division, Thiokol Corp. (Danvers, Mass.). This material was unsuitable for HPLC applications because of the very small particle sizes present, high back pressure, and therefore was not studied further. Two different particle size anion exchange resins were utilized for the in-house prepared borohydride resin, these being AG 1-X8 (less than 400 mesh) and Aminex A-25 (17.5 ± 2 um particle size). Both of these polymeric resins were obtained from Bio-Rad

Laboratories (Richmond, Calif.). Such resins are copolymers of styrene-divinyl benzene, in varying ratios depending on the degree of cross-linking desired.

HPLC solvents were obtained as follows: 1) methanol was Omnisolv from MCB Manufacturing Chemists, Inc. (Gibbstown, N.J.); 2) acetonitrile was also Omnisolv; and 3) water was from J.T. Baker Chemical Co. (Phillipsburg, N.J.).

The various chemicals and reagents utilized were obtained from commercial suppliers, of the highest purity commercially available, and were used as received, without further purification. These were obtained as follows: sodium borohydride (Aldrich Chemical Co., Milwaukee, Wisc.); acetophenone (Aldrich); benzhydrol (Aldrich); 2-naphthaldehyde (Aldrich); 2-naphthol (Aldrich); 1-indanone (Aldrich); 1-indanol (Aldrich); salicylamide (Aldrich); cinnamoyl chloride (Aldrich); benzyl alcohol (Aldrich); trans-cinnamaldehyde (Aldrich); cinnamyl alcohol (Aldrich); benzaldehyde (J.T. Baker Co.); benzophenone (Fisher Sci. Co., Medford, Mass.); aspirin (Sigma Chemical Co., St. Louis, Mo.); phenacetin (Sigma); p-nitrobenzaldehyde (Sigma); p-nitrobenzaldehyde (Aldrich); p-nitrobenzoyl chloride (Aldrich); cinnamon (R.T. French Co., Rochester, N.Y.); and Lavoris mouthwash (Osco Drugs, Boston, Mass.).

Apparatus

The HPLC system utilized for most of these studies consisted of a Waters U6K syringe loading injection valve (Waters Associates, Inc., Milford, Mass.), a Waters 6000A solvent delivery system (pump), a Waters Model 480 variable wavelength UV-VIS detector, and a Houston Omniscribe dual pen recorder (Houston Instruments, Inc., Houston, Texas). A number of reversed phase HPLC columns were used, but in general, a 10um generic C_{18} type column was used, 25-cm x 4.6-mm i.d. The dummy column and solid phase reactor column were prepared using glass lined stainless steel tubing, 6-cm x 4.6-mm i.d., from Alltech Associates (Deerfield, Ill.). All dummy, solid phase reactor, and analytical end column fittings were zero dead volume (Cambridge Valve & Fitting, Cambridge, Mass.). Wherein both the dummy column and the solid phase reactor were both on-line simultaneously, individual injections were switched to either the dummy or SPR via a Rheodyne Model 7000 switching valve (Rheodyne Corp., Berkeley, Calif.). The switching valve was located just after the HPLC injection valve and before the dummy and SPR columns, all of which was located just before the analytical column.

Methods

In all of these studies with the polymeric borohydride resin, standards of all organic compounds being analyzed were injected as solutions in the mobile phase or neat acetonitrile. Such standard solutions, in known amounts,

were generally injected in 20 ul aliquots, first onto a combination of the dummy column plus analytical column, and then onto the polymeric borohydride reduction column (SPR) and the same analytical column. The dummy column consisted of the commercial anion exchange resin, usually the Aminex A-25, in its original chloride (Cl^-) form. The polymeric borohydride resin in the solid phase reactor and the dummy column were both slurry packed, at pressures of about 2000 psi. During normal HPLC operations, both the dummy and solid phase reactor columns were stable at 1500-2000 psi. It is possible that these packings are stable to higher back pressures, when used in the pre-analytical column mode, but we have not had to determine maximum pressure stabilities. All retention times on both dummy and solid phase reactor columns, pre-analytical column, were determined by duplicate or triplicate injections of the analyte of interest along with expected reduction product(s), the alcohols. In all cases, with various mixtures of mobile phases, the retention times of the alcohols on both the dummy and solid phase reactor columns agreed very well (± 5% or less).

In the case of the analysis for cinnamaldehyde in cinnamon, this was done by first extracting the cinnamon, in a known amount, with a small volume of methanol (1-2 mls), filtering this extract, and then immediately injecting the extract onto both the dummy and SPR columns, pre-analytical mode. The identification of cinnamaldehyde and cinnamyl alcohol was based on measured retention times. Retention times for all compounds were based on the HPLC chromatograms and an external automatic timer started at the point of injection and measured at the point of maximum peak height(s).

The polymeric borohydride solid phase reactor was prepared according to a procedure available from Ventron Corp., Division of Thiokol Corp. (Beverly, Mass.) (20, 21). This is a standard method for preparing the polymeric borohydride resin, and consists of several distinct steps. These successive steps of preparation are: 1) pre-purification of the commercial chloride-form resin (Aminex A-25); 2) conversion to the borohydride resin form: 3) post-purification of the borohydride resin; and 4) drying of the final borohydride resin and analysis for boron content and/or active borohydride content *via* titration. Elemental analyses for boron content were performed at Galbraith Laboratories (Knoxville, Tenn.), and the active borohydride content determinations were done in-house, using an iodine titration procedure described elsewhere (22, 23). More specific experimental conditions for the preparation of the borohydride resin are available from Ventron Corporation (21).

RESULTS AND DISCUSSION

Figure 1 indicates some typical examples of aldehydes and ketones that have been utilized with the polymeric borohydride SPR. We have studied other types of carbonyl derivatives, such as amides, acid chlorides, and esters, as well as N-nitroso compounds, aryl halides, and certain drugs, Table 3. The overall analytical results utilizing this borohydride SPR are indicated in Table 3, wherein NR signifies that no reaction was observed. Chemical reductions were determined by injecting a mixture of the carbonyl compound and its expected (known) reduction product (alcohol), onto the dummy plus analytical column. When mobile phase conditions were obtained that yielded baseline resolution of the two compounds, then the same mixture was injected onto the SPR plus analytical column, using the same mobile phase and flow rate. The disappearance of the starting aldehyde and the concomitant increase in peak height/area for the expected alcohol product were then used as sufficient evidence for chemical reduction. At the same time, each carbonyl compound was injected alone onto the dummy plus analytical column followed by another injection onto the SPR plus analytical column. The formation of the expected alcohol reduction product then became even clearer, if indeed chemical reduction had taken place. All of these analyses have been done at room temperature, but in the case of certain ketones, elevated temperatures of 40-50^{o}C were utilized, in an effort to force some degree of chemical reduction. However, in no case, even with elevated reaction temperatures, have we been able to effect any on-line polymeric borohydride reductions except in the case of the aldehydes, Table 3. This degree of selectivity is what one might have expected based on the known synthetic organic reports utilizing this particular borohydride resin.

A typical SPR column contains about 1.6 g of the polymeric borohydride resin, but obviously this could be changed just by changing the dimensions of the glass lined stainless steel cartridge. Knowing the exchange capacity of the Aminex A-25 resin to be about 1.4 meq/ml, and the density of the resin to be about 0.8 g/ml, the total exchange capacity of the resin in a typical SPR column would then be 2.8 meq. This converts to about 38.6 mg of active borohydride present within a typical on-line reduction column. We have determined the actual borohydride content present in these columns using an accepted titration procedure (22, 23). For two different preparations of the polymeric borohydride resin, the borohydride content was 1.54 to 2.30 % (as borane), which is equal to 24.64 to 36.8 mg in the SPR column. The actual level of incorporated borohydride (borane) in any given resin preparation will depend on how the particular resin batch is prepared. However, even at the lower level indicated above (24.64 mg), this particular SPR would be active for several

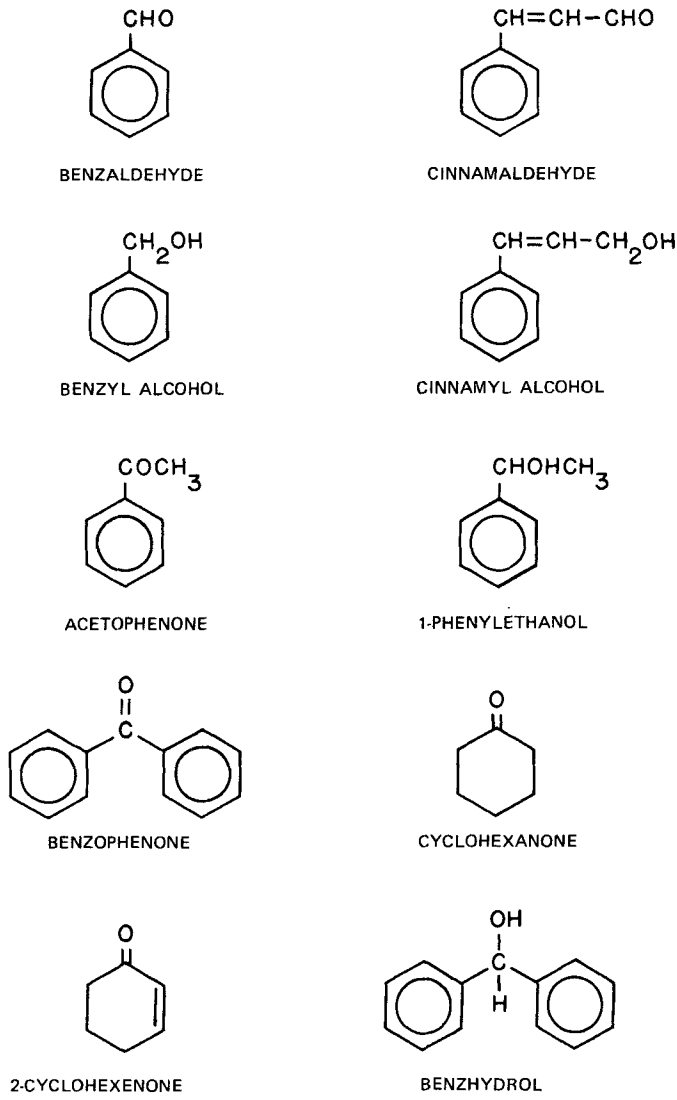

Figure 1. Typical aldehydes and ketones studied via solid phase reactors in HPLC.

TABLE 3

CLASSES OF COMPOUNDS STUDIED VIA POLYMERIC BOROHYDRIDE IN HPLC[a]

CLASS OF COMPOUNDS AND MEMBERS	POLYMERIC BOROHYDRIDE IN HPLC
1. ALDEHYDES	
benzaldehyde	100%
cinnamaldehyde	100%
p-nitrobenzaldehyde	100%
2-naphthaldehyde	100%
2. KETONES	
benzophenone	NR[b]
acetophenone	NR
1-indanone	NR
3. AMIDES	
salicylamide	NR
4. ACID CHLORIDES	
cinnamoyl chloride	NR
o-nitrobenzoyl chloride	NR
5. ARYL HALIDES	
chlorobenzene	NR
6. DRUGS	
acetylsalicylic acid (aspirin)	NR
acetophentidine (phenacetin)	NR
7. N-NITROSO	
N-nitrosodiphenylamine	NR
N-nitrosodiethylamine	NR
1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU)	NR

a. Analytical conditions used a polymeric borohydride reaction column alone, 6-cm x 4.6-mm i.d., with mobile phase of 30% HOH/MeOH at 1.0 ml/min, no analytical column in-line. Comparisons made with dummy column under identical analytical conditions.

b. NR = no reaction observed = no change in peak height for starting compound.

hundred individual analyses. The SPRs prepared in-house have now been effective for several months without showing any signs of decreased activity or reduction effectiveness. Their use has involved several hundred individual injections of various carbonyl compounds, without any discernible loss of reductive efficiency or capacity. This suggests that SPRs in HPLC can have unusually long life-times, and that they can be utilized with both standards and real world samples for hundreds of individual analyses. At the trace levels of analytes, these same SPRs should be active for perhaps thousands of analyses.

With regard to the absolute amounts or concentrations of aldehydes that can be reduced in real-time, on-line, with this particular SPR, we have investigated benzaldehyde and cinnamaldehyde. It is possible to have 100% reduction of both aldehydes at constant volume injected or with variable injection volumes. In the case of benzaldehyde, injections from 5 ng to 50 ug in 20 ul injections are all 100% reduced to benzyl alcohol. Cinnamaldehyde in the range of 5 ng to 5 ug is also fully reduced under the same conditions. All of these analyses were done using a conventional 10 um C_{18} reversed phase column together with a mobile phase of 30% MeOH/HOH at a flow rate of 1.0 ml/min. The polymeric borohydride column was in the pre-analytical column orientation, with UV detection at 254nm. In each case, the disappearance of the starting aldehyde occurred concomitant with the appearance of the expected alcohol reduction product.

Reversed phase HPLC utilizes various mixtures of MeOH/HOH or ACN/HOH, although other combinations of organic modifiers have at times been utilized. We have used these solvent combinations in order to determine the compatibility and effectiveness of the polymeric borohydride resin towards benzaldehyde and cinnamaldehyde. Table 4 summarizes the retention times (t_r) and percent reductions observed as a function of varying the ratio of MeOH/HOH from 100% MeOH to 30% MeOH/HOH. In order to reduce the overall retention times for these two aldehydes with 30% MeOH/HOH, we used a C_8 analytical column instead of the C_{18} column used with the other solvent mixtures. These reductions are also effected using 100% HOH as the mobile phase, although such studies required the omission of the analytical column because of very long retention times.

Table 5 summarizes the retention times and percent reductions obtained for the same aldehydes, but now using various mixtures of ACN/HOH, going from 80% ACN/HOH down to 30% ACN/HOH. Again, one observes 100% reduction of both aldehydes regardless of the ACN/HOH mobile phase composition. We have also studied, to a limited extent, the use of EtOH/HOH mixtures as the HPLC mobile phase, and all of the ratios thus far tried have resulted in 100% reduction of the two aldehydes. Hence, for precisely those HPLC mobile phase

TABLE 4

RETENTION TIMES AND PERCENT REDUCTIONS OF AROMATIC ALDEHYDES VIA POLYMERIC REDUCING RESIN[a] AS A FUNCTION OF REVERSED PHASE MOBILE PHASE SOLVENT COMPOSITIONS

MOBILE PHASE MAKE-UP	100% MeOH[b]		70% MeOH/HOH[b]		50% MeOH/HOH[b]		30% MeOH/HOH[c]	
ANALYTE OF INTEREST	t_r(sec)	% redn.	t_r(sec)	% redn.	t_r (sec)	% redn.	t_r(sec)	% redn.
BENZALDEHYDE	222	100	322	100	654	100	1395	100
BENZYL ALCOHOL	218	----	284	----	488	----	882	----
CINNAMALDEHYDE	219	100	359	100	1010	100	3078	100
CINNAMYL ALCOHOL	218	----	347	----	946	----	2670	----

a. Polymeric reduction resin prepared from Aminex A-25 with ionically attached reducing agent, column size 6.0-cm x 4.6-mm i.d., in pre-analytical column mode, glass-lined st. steel SPR.
b. HPLC reversed phase C-18 column, 25-cm x 4.6-mm i.d., 10um packing, flow rate 1.0 ml/min, UV detection at 254nm.
c. HPLC reversed phase C-8 column, 25-cm x 4.6-mm i.d., 10um packing, flow rate 1.0 ml/min, UV detection at 254nm.

TABLE 5

RETENTION TIMES AND PERCENT REDUCTIONS OF AROMATIC ALDEHYDES VIA POLYMERIC REDUCING RESIN AS A FUNCTION OF REVERSED PHASE MOBILE PHASE SOLVENT COMPOSITION (ACN/HOH)[a]

MOBILE PHASE COMPOSITION	80% ACN/HOH[b]		50% ACN/HOH[b]		30% ACN/HOH[c]	
ANALYTE OF INTEREST	t_r(sec)	% REDN.	t_r(sec)	% REDN.	t_r(sec)	% REDN.
BENZALDEHYDE	234	100	414	100	818	100
BENZYL ALCOHOL	212	---	282	---	467	---
CINNAMALDEHYDE	242	100	503	100	1289	100
CINNAMYL ALCOHOL	225	---	373	---	896	---

a. Polymeric reducing resin was prepared from Aminex A-25 with ionically attached borohydride, column size 6.0-cm x 4.6-mm i.d., in pre-analytical column mode, glass lined stainless steel tube.
b. HPLC reversed phase C-18 column, 25-cm x 4.6-mm i.d., 10 um packing material, flow rate 1.0 ml/min, UV detection at 254nm.
c. HPLC reversed phase C-8 column, 25-cm x 4.6-mm i.d., 10 um packing material, flow rate 1.0 ml/min, UV detection at 254nm.

compositions of most interest in reversed phase separations, it is indeed possible to effect complete reductions for at least two representative aromatic aldehydes. It is expected that other aromatic and aliphatic aldehydes should exhibit these same overall reductions with the same types of HPLC mobile phases, as above.

As typical illustrative chromatograms for the on-line, real-time polymeric borohydride reductions of aldehydes in reversed phase HPLC, Figure 2 indicates such a study with benzaldehyde. Figure 2A is the HPLC-UV chromatogram for both benzaldehyde and benzyl alcohol injected together, with a mobile phase of 50% HOH/MeOH at a flow rate of 1.0 ml/min, and with the dummy column before the analytical column. Approximately equal amounts of each compound were injected as a mixture. Retention times were determined by injecting each standard separately under identical HPLC conditions. Figure 2B indicates the injection of this same mixture onto the polymeric borohydride SPR operating before the analytical column, again with UV detection at 254nm. It is immediately clear that the benzaldehyde is fully reduced under such analytical conditions, and that there is an increased peak height and area for the expected reduction product, benzyl alcohol.

Figure 3 indicates a situation wherein cinnamyl alcohol and cinnamaldehyde are not fully resolved when co-injected onto the dummy column plus analytical column. In Figure 3A, these two compounds are resolved on the analytical column alone, using 30% HOH/MeOH at 1.0 ml/min. However, in Figure 3B, the same mixture injected onto dummy plus analytical column leads to a single, somewhat broader peak. Finally, Figure 3C is an injection of the same mixture onto the polymeric borohydride SPR plus analytical column. Although the two compounds are not resolved in either 3B or 3C, the increase in peak height and peak area in Figure 3C clearly indicates that a reduction of the aldehyde must have occurred. Thus, wherein the reduction product has a greater extinction coefficient (Epsilon) than the starting material at a particular wavelength, the resultant increase in peak height and peak area can be used to demonstrate chemical reduction in unresolved mixtures. Obviously, wherein the reduction product has a smaller extinction coefficient, this type of reaction would result in a decreased peak height or complete disappearance of the starting material and no product peak formation. It is naturally of interest that such chemical reductions can, in certain instances, lead to increases in peak heights and therefore improved detection limits for the starting aldehyde.

Two additional illustrative examples for these same reductions of the above two aldehydes are indicated in Figures 4 and 5. Figure 4A is the injection of both benzaldehyde and benzyl alcohol onto the dummy and analytical

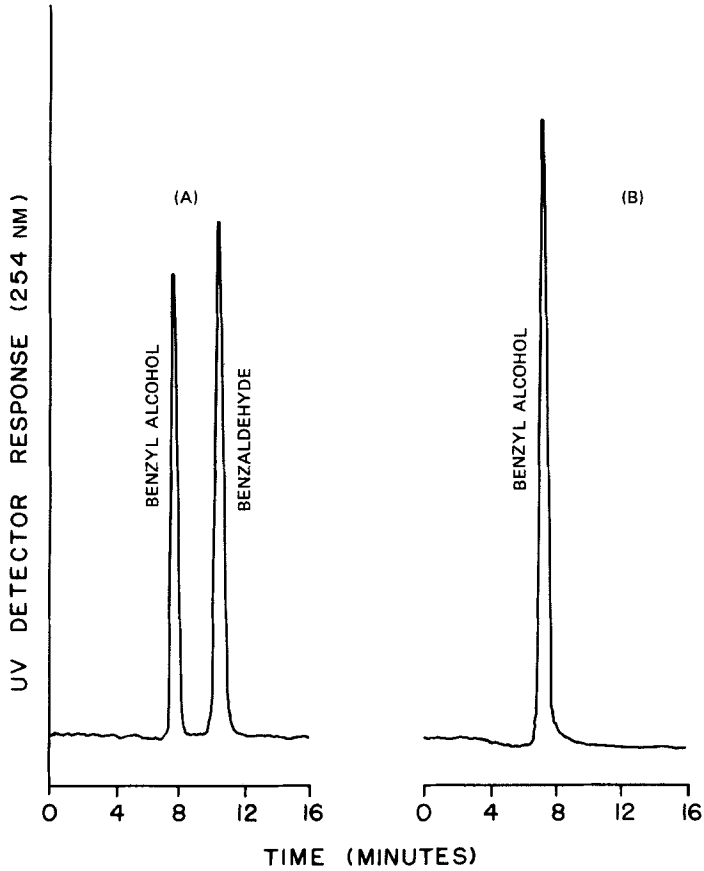

Figure 2. HPLC-UV chromatograms of benzaldehyde and benzyl alcohol using a mobile phase of 50% HOH/MeOH at a flow rate of 1.0 ml/min with the polymeric reduction column in pre-column mode: (A) dummy column in-line before analytical RP column; (B) polymeric reduction column in-line before analytical RP column. Dummy column in chloride form.

column using 50% ACN/HOH at 1.0 ml/min with UV detection at 254nm. Figure 4B is the same injection onto the polymeric reduction SPR plus analytical column, with the same mobile phase and flow rate. Again, there is complete disappearance of the benzaldehyde peak and a somewhat increased peak height for the expected reduction product, benzyl alcohol. In Figure 5A, both cinnamaldehyde and cinnamyl alcohol are injected together onto the dummy plus analytical column, again using 50% ACN/HOH as the mobile phase. In Figure 5B,

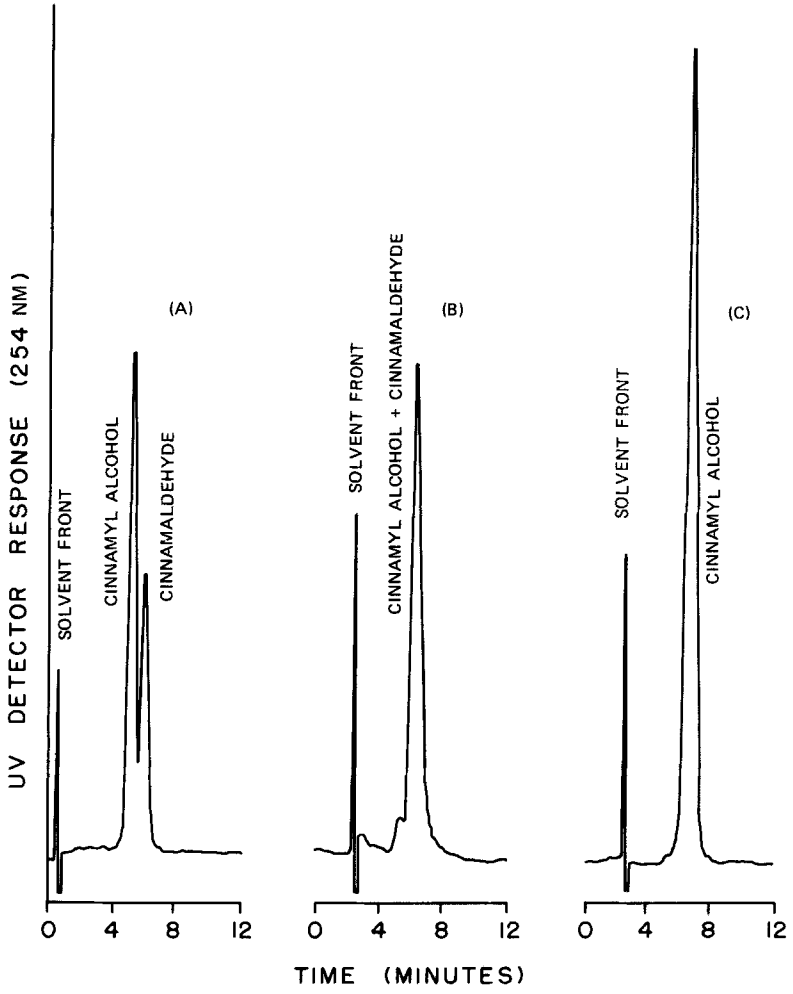

Figure 3. HPLC-UV chromatograms of cinnamaldehyde and cinnamyl alcohol using a mobile phase of 30% HOH/MeOH at flow rate of 1.0 ml/min with polymeric reduction column in pre-column mode: (A) analytical column (C-18) alone; (B) dummy column in-line before analytical column; (C) polymeric reduction column in-line before analytical column. Dummy column in chloride form in all cases.

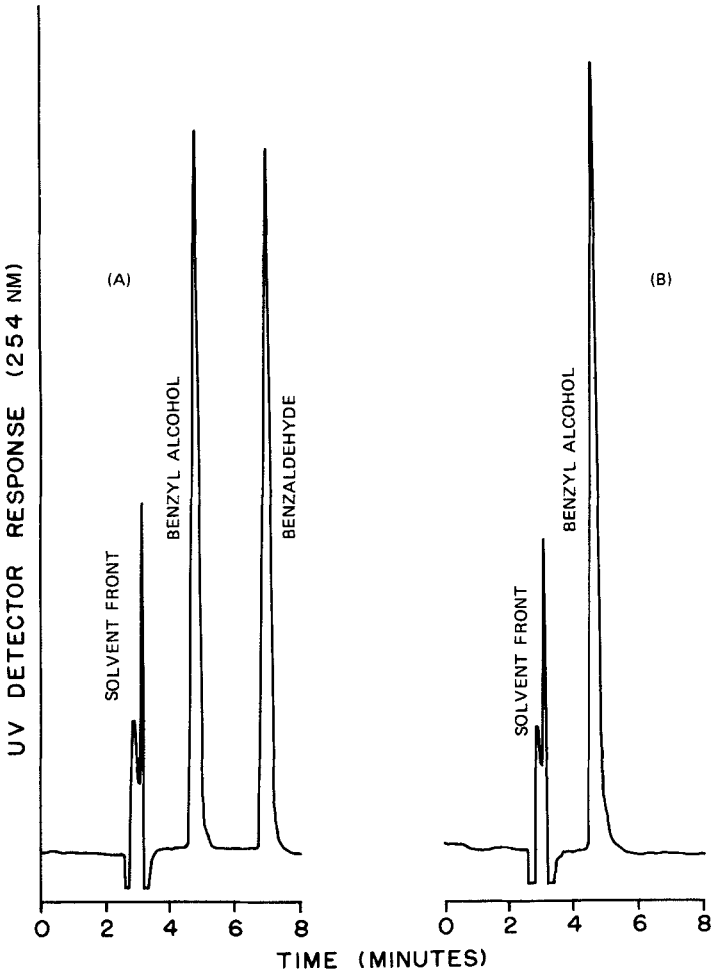

Figure 4. HPLC-UV chromatograms of benzaldehyde and benzyl alcohol with polymeric reduction column in pre-column mode and RP C-18 analytical column with mobile phase of 50% ACN/HOH at 1.0 ml/min: (A) mixture of compounds with dummy column and analytical column; (B) same mixture of compounds with polymeric reduction column and analytical column. Dummy column in chloride form in all cases.

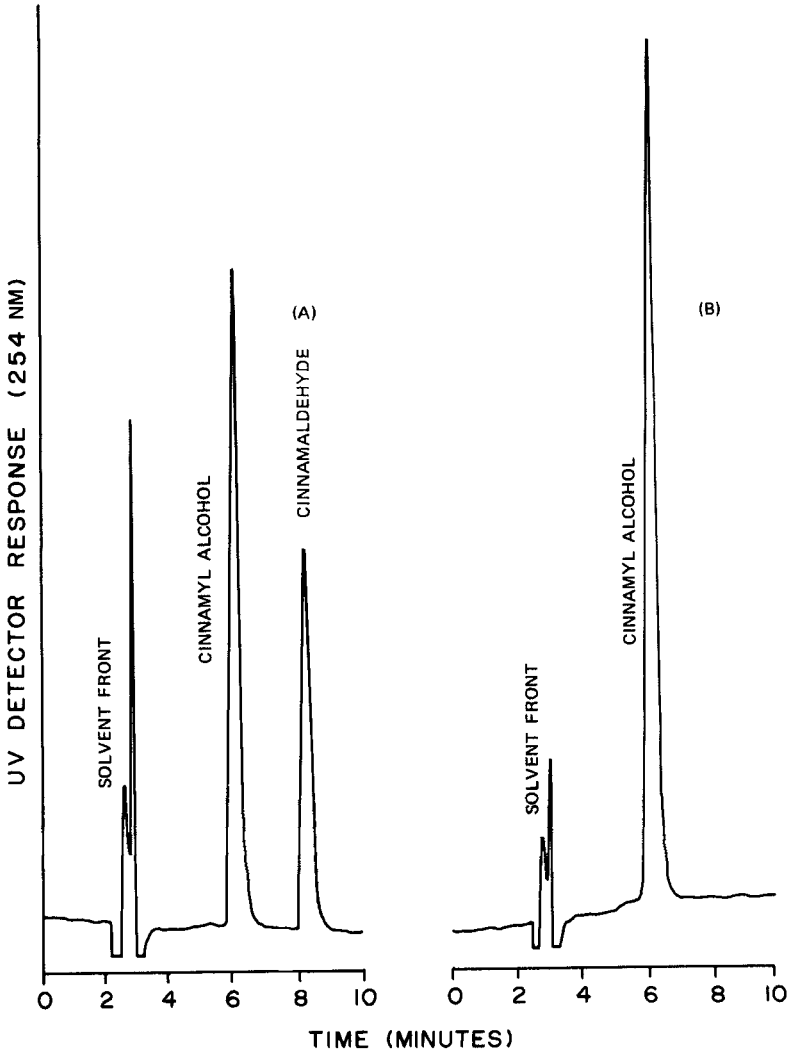

Figure 5. HPLC-UV chromatograms of cinnamaldehyde and cinnamyl alcohol with polymeric reduction column in pre-column mode and RP C-18 analytical column and mobile phase of 50% ACN/HOH at 1.0 ml/min: (A) mixture of compounds on dummy column and analytical column; (B) mixture of same compounds on polymeric reduction column plus analytical column.

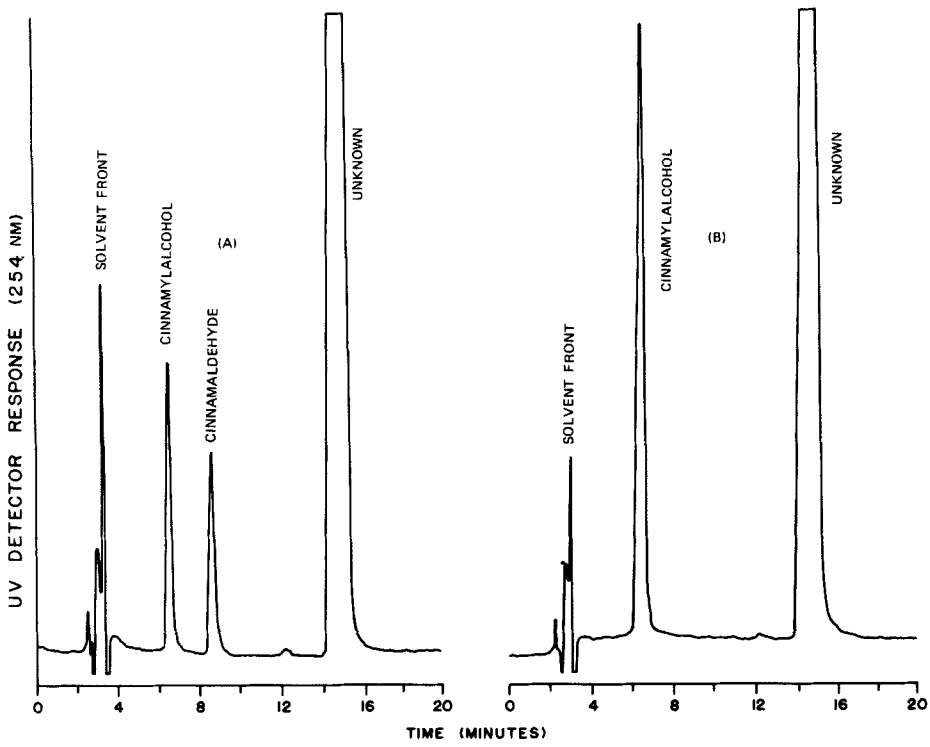

Figure 6. HPLC-UV chromatograms of methanolic extract of commercial sample of cinnamon for the determination of cinnamaldehyde. HPLC conditions used a C-18 RP analytical column with mobile phase of 50% ACN/HOH at 1.0 ml/min. UV detection at 254nm. (A) polymeric dummy column plus analytical column; (B) polymeric borohydride reducing column in-line before analytical column.

the same mixture is injected onto a combination of the SPR plus analytical column, and once again there is complete disappearance of the aldehyde peak and a greatly increased peak height for the cinnamyl alcohol formed.

We have now applied the above approaches for the trace analysis of cinnamaldehyde in two consumer products. In the first application, we have extracted a known amount of the commercial spice, cinnamon, with a small, known volume of methanol. After filtration of this extract, it was injected onto the dummy plus analytical column first, Figure 6A, and both cinnamaldehyde and cinnamyl alcohol were both evident. Figure 6B is an injection of the same

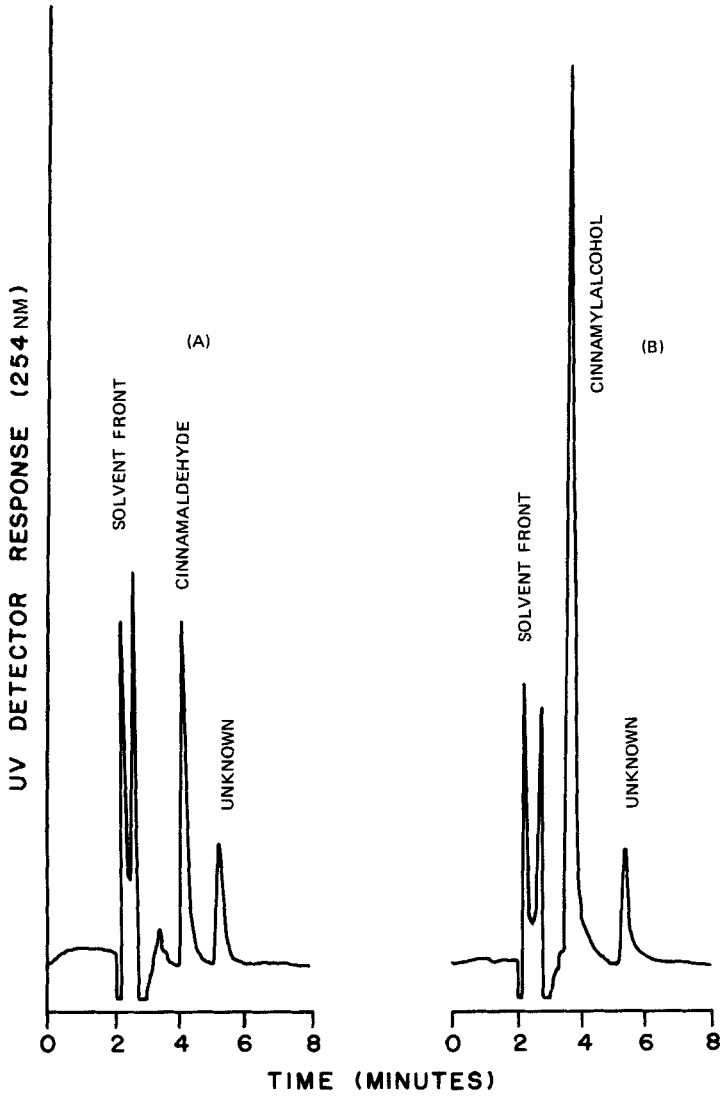

Figure 7. Analysis for cinnamaldehyde in Lavoris mouth wash via HPLC-solid phase derivatization methods. LCUV chromatograms of mouth wash diluted with MeOH: (A) polymeric dummy column in-line before analytical column; (B) polymeric reducing column in-line before analytical column. LC used C-18 analytical column, 10um, 25-cm x 4.6-mm i.d., plus mobile phase of 25% HOH/ACN at 1.0 ml/min, UV at 254nm.

extract onto the polymeric borohydride SPR plus analytical column, using the mobile phase of 50% ACN/HOH at 1.0 ml/min flow rate. There is a complete disappearance of the peak due to cinnamaldehyde with a concomitant increase in the peak height for the expected product, cinnamyl alcohol. Other peaks from the cinnamon extract are apparent, but they do not interfere in the analysis and confirmation for cinnamaldehyde.

One other application of these methods is demonstrated in Figures 7A and 7B, wherein a sample of Lavoris mouthwash was first diluted 500 fold with methanol. Figure 7A is the LCUV chromatogram of this sample on the dummy plus analytical column, using 25% HOH/ACN at 1.0 ml/min flow rate. Only a peak perhaps due to cinnamaldehyde is apparent, although there may be a small amount of cinnamyl alcohol present as well. In Figure 7B, with the same sample injected onto the SPR plus analytical column, all of the suspected cinnamaldehyde peak has disappeared, and a new peak with the retention time of authentic cinnamyl alcohol has appeared. Clearly, these very approaches could easily be utilized for cinnamaldehyde or other aldehydes present at trace levels in foods, beverages, water, environmental samples, industrial products or raw materials, consumer products, and so forth.

CONCLUSIONS

We have demonstrated the applicability and usefulness of certain polymeric borohydride reducing resins for the highly selective and specific chemical reduction, on-line, of various aldehydes. These solid phase reactors can be used with a wide variety of reversed phase solvents and conventional columns, flow rates, and detectors. They perform selective carbonyl reductions in aldehydes alone, with no changes in the HPLC flow rates, injection volumes, or instrument operating temperature. Thus, these particular SPRs are fully compatible with all modern day HPLC instruments, and they require no additional modifications in either the equipment or analytical approaches. The only additional work required is the switching of the dummy column for the SPR, perhaps via a permanently mounted switching valve after the injection valve, followed by single or duplicate injections of the same sample solution in each arrangement. However, there is no additional sample work-up or preparation required, no additional derivatization equipment or instrumentation, no mixing of HPLC eluents and derivatization solutions, no incubations of derivatization solution with eluents, no added hold-up of the sample within the chemical reactor, and in general, a much simpler and more direct approach to on-line, real-time, chemical derivatizations.

It is our belief that these approaches should find wide acceptance and applications for a very large number of samples. Although these initial results indicate a high selectivity for aldehydes, with no reactions yet observed for ketones, acid chlorides, esters, etc., it is indeed possible that yet other, already described or to be developed, polymeric or supported reduction reagents may exhibit different degrees of analyte selectivity. We are, at present, investigating yet other possible supported and/or polymeric reducing reagents, and these results indeed indicate that other apporaches provide different chemical reactivities for aldehydes and other classes of carbonyl derivatives. It should also be clear that we are not limited in these SPR approaches to only chemical reductions, but that virtually all types of chemical reactions and derivatizations may yet be possible with either supported or polymeric chemical reagents and derivatization systems. We are now pursuing a number of these most useful and interesting derivatization approaches in HPLC.

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24. Abbreviations used: HPLC = high performance liquid chromatography; UV = ultraviolet detection; FL = fluorescence detection; EC = electrochemical detection; MeOH = methanol; ACN = acetonitrile; RP = reversed phase; HOH = water; EtOH = ethanol.