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Elimination and reduction of chlorinated solvents in preparative liquid chromatography

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ABSTRACT

Increasing safety concerns and environmental legislation have mandated the reduction and eventual elimination of chlorinated solvents. Therefore, the Preparative Chromatography Group at Searle is working to eliminate the use of chlorinated solvents. Three areas of investigation to meet this goal are under way: (1) develop replacement solvents, (2) reduce the percentage of chlorinated solvents and (3) develop alternative sample application techniques. This paper demonstrates the progress that has been made in all three areas.

INTRODUCTION

Chlorinated solvents such as chloroform, methylene chloride and 1,1,2-trichloro-1,2,2-trifluoroethane are very useful for the purification of low-molecular-mass compounds [1–3]. This is due to the unique chromatographic selectivity and the extreme solubility that many compounds exhibit in chlorinated solvents. Additional benefits of chlorinated solvents include low flammability, low viscosity and high volatility, all of which are desirable for preparative chromatography [4]. However, chlorinated solvents are known central nervous system depressants and are suspected carcinogens [5–8]. In addition, environmental legislation, such as the Montreal Protocol and Clean Air Act, mandates a phase-out on all substances that deplete the ozone layer [9,10]. These concerns have prompted Searle chromatographers to develop alternatives to the use of chlorinated solvents in preparative liquid chromatography. Three main areas will be discussed: (1) replacement of chlorinated solvents, (2) reduction of the percentage of chlorinated solvent in the mobile phase and (3) alternative sample application techniques. This paper reports on the progress that the Preparative Chromatography Group at Searle has made towards eliminating the use of chlorinated solvents.

EXPERIMENTAL

Equipment

Numerous preparative chromatographic systems were used and obtained from a variety of sources. Preparative columns were obtained from MODcol (St. Louis, MO, USA) and YMC (Wilmington, NC, USA).

Materials

TLC separations were achieved on Merck (Darmstadt, Germany) silica gel 60 F_{254} TLC plates or Woelm silica gel 60 F_{254} TLC plates purchased from Analtech (Newark, DE, USA). The bulk packings were Merck silica gel 60, 40–63-μm, 60-Å irregular silica gel from EM Science (Cherry Hill, NJ, USA) and ICN adsorbent 32–63-μm, 60-Å irregular silica gel from ICN Biomedicals (Cleveland, OH, USA). All

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samples for purification were synthesized in the laboratories of Searle (Skokie, IL, USA). The solvents and reagents were of analytical-reagent grade or better and obtained from a variety of sources.

RESULTS AND DISCUSSION

The long-term goal of the Preparative Chromatography Group is the total elimination of the use of chlorinated solvents. To achieve this goal, two approaches have been followed: (1) the elimination of chlorinated solvents whenever possible and (2) a reduction in the volume of chlorinated solvents when total elimination is not possible.

Elimination of chlorinated solvents

Preparative chromatography has been performed at Searle for over 30 years. During this time, numerous purification methods and structure–solvent relationships using chlorinated solvents have been developed. Alternatives to chlorinated solvents needed to be developed which could provide good and predictable separations on a preparative scale. In the past 3 years, many solvents have been investigated. TLC analysis and purification of more than 100 samples has shown two solvents, ethyl acetate and methyl *tert.*-butyl ether, to have similar chromatographic characteristics to methylene chloride. These solvents can replace a chlorinated solvent, but do not produce exactly the same sample profile by TLC. Fig. 1 shows a sample analyzed by TLC with ethyl acetate and methyl *tert.*-butyl ether directly substituted for methylene chloride. The order of the components varies, as does the degree of separation, but the main component is sufficiently separated to allow a purification by preparative chromatography. Whereas the direct substitution of ethyl acetate for methylene chloride gives approximately the same R_F, the substitution of methyl *tert.*-butyl ether results in a lower R_F. The increased use of ethyl acetate and methyl *tert.*-butyl ether has reduced the number of purifications that require chlorinated solvents.

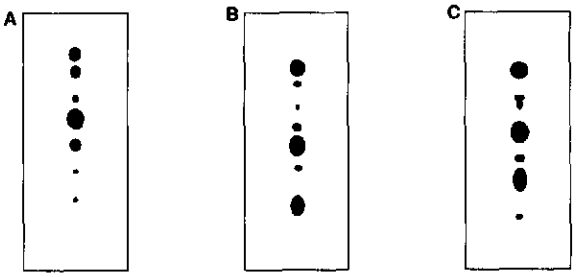

Fig. 1. Thin-layer chromatographic analysis using methylene chloride, methyl *tert.*-butyl ether and ethyl acetate. Solvent system: (A) methylene chloride–methanol–ammonia solution (85:14:1); (B) methyl *tert.*-butyl ether–methanol–ammonia solution (85:14:1); (C) ethyl acetate–methanol–ammonia solution (85:14:1). Detection, *tert.*-butyl hypochlorite–starch–potassium iodide; TLC adsorbent, Merck silica gel 60 F_{254}.

Reduction of chlorinated solvents

Existing methods for the purification of many classes of pharmaceutical compounds often utilize chlorinated solvents. If elimination of the chlorinated component from a method cannot be accomplished in a reasonable time frame, then a reduction in the volume of chlorinated solvent is investigated to carry out the separation. Two reduction techniques are (1) to lower the percentage of chlorinated solvent in the mobile phase and (2) to develop a purification scheme that utilizes multiple purifications. The objective of these techniques is the use of the smallest volume of chlorinated solvent.

If a chlorinated solvent is required to achieve the separation, an attempt is made to reduce the percentage of chlorinated solvent. Often a portion of the chlorinated solvent can be substituted with heptane. In this way a smaller volume of chlorinated solvent is used, and the beneficial characteristics associated with the chlorinated solvent are still employed. An example of this approach is shown in Fig. 2.

Another technique for the reduction of chlorinated solvents is the use of multiple purifications to achieve the separation. If a complex sample contains many components, two purifications are performed to achieve the desired separation. An example of this is shown in Fig. 3. The first purification, which uses a non-chlorinated mobile phase, is used to remove unwanted components. The second purification uses a chlorinated mobile phase to complete the separa-

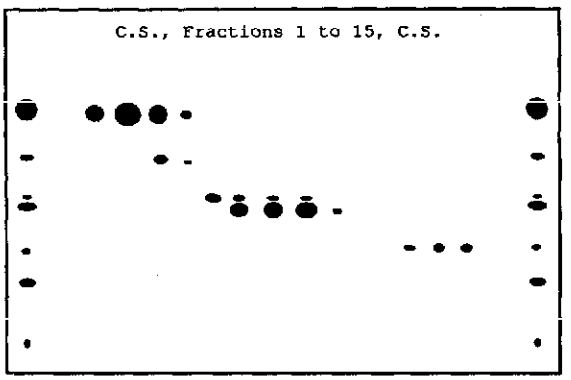

Fig. 2. Example of reduction of percentage of chlorinated solvent in purification mobile phase. TLC solvent system, ethanol–methylene chloride (5:95); TLC adsorbent, Merck silica gel 60 F_{254}; detection, UV absorption at 254 nm. C.S. is sample prior to chromatography. Purification mobile phase: fractions 1–7, ethanol–methylene chloride–heptane (3:24:73); fractions 8–15, ethanol–methylene chloride–heptane (5:40:55); 9.8 g of material was purified on a 50 cm × 50 mm I.D. column; Flow-rate, 500 ml/min.

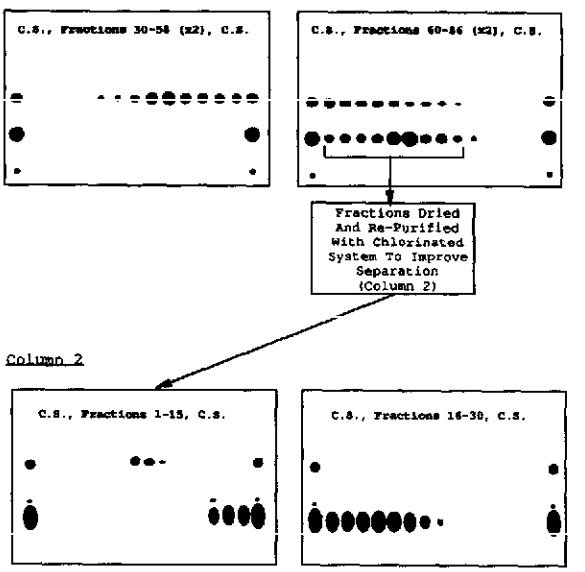

Fig. 3. Multiple column strategy approach for reducing the volume of chlorinated solvent required for purification. Mobile phase and column dimensions: column 1, ethyl acetate–heptane, 50 cm × 2.54 cm I.D.; column 2, ethyl acetate–methylene chloride, 25 cm × 2.54 cm I.D. Sample size and flow-rate; column 1, 4.1 g, 56 ml/min; column 2, 1.5 g, 28 ml/min. Column 1 TLC conditions: solvent system, ethyl acetate–heptane (40:60); TLC adsorbent Woelm silica gel 60 F_{254}; detection, iodine–UV absorption at 254 nm. Column 2 TLC conditions: solvent system, ethyl acetate–methylene chloride (40:60); TLC adsorbent, Woelm silica gel 60 F_{254}; detection, iodine–UV absorption at 254 nm.

tion. After the first purification, the sample size is reduced so that a smaller column can be used for the second purification. The smaller column results in a subsequent decrease in the volume of chlorinated solvent used.

In lieu of providing many examples, the data in Table I show a comparison of the number of purifications utilizing chlorinated solvents over the past 3 years. The decrease from 1991 to 1993 provides evidence that the practice of solvent replacement has become routine and efficient.

Alternative sample application techniques

Sample solubility problems have been encountered as chlorinated solvents have been reduced and eliminated from the purifications. Alternative sample application techniques have been utilized when limited solubilities are encountered. The alternative techniques for application of insoluble samples include dissolution in small volumes of chlorinated solvent, pre-adsorption of the sample on the stationary phase, direct wet sample application and solid injection.

A sample with limited solubility in the mobile phase can be dissolved in a small volume of chlorinated solvent and injected for purification. Owing to the high solvating power of chlorinated solvents, only a small volume is needed to apply the sample to the column. An example of the successful application of this technique is shown in Fig. 4. One of the potential drawbacks of dissolving the sample in a solvent other than the mobile phase is sample breakthrough. An example of this is shown in Fig. 5. In sample break-

TABLE I

SAMPLE PURIFICATIONS REQUIRING CHLORINATED SOLVENT

Year	Percentage of samples purified using chlorinated solvents[a]
1991	34
1992	22
1993	7

[a] Data are for the first quarter of each year.

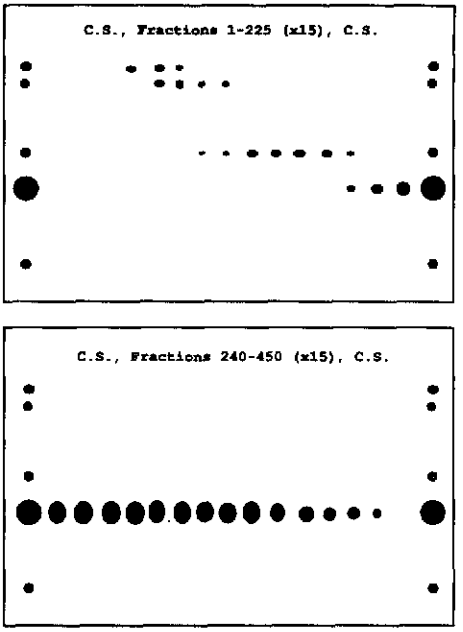

Fig. 4. Example of sample dissolution in chlorinated solvent. Mobile phase, ethyl acetate–heptane (30:70). Sample dissolved in 50 ml of methylene chloride; 20 g of material were purified on a 50 cm × 47.5 mm I.D. column; flow-rate, 20 ml/min; TLC solvent system, ethyl acetate–heptane (50:50); TLC adsorbent, Merck silica gel 60 F_{254}; detection, UV absorption at 254 nm.

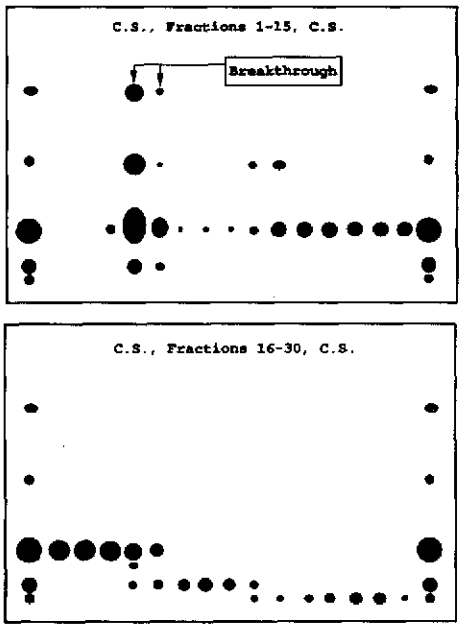

Fig. 5. Example of sample breakthrough caused by dissolution in chlorinated solvent. Mobile phase, ethyl acetate–heptane (5:95). Sample dissolved in 50 ml of chloroform; 17.69 g of sample purified on a 50 cm × 4.45 cm I.D. column; flow-rate, 180 ml/min. TLC solvent system, ethyl acetate–heptane (5:95); TLC adsorbent, Woelm silica gel 60 F_{254}; detection, iodine–UV absorption at 254 nm.

through, part of the sample does not adsorb on the stationary phase and elutes with no separation taking place. To minimize this effect, the mobile phase system can be adjusted to increase the capacity factor (k') of the desired component.

Another technique for the application of insoluble samples is pre-adsorption of the sample on the stationary phase [11–13]. In this process the sample is dissolved in any solvent and combined with the stationary phase. The solvent is then evaporated to produce a dry, powdered matrix which is packed into a sample column. With this technique the risk of breakthrough is eliminated. The primary drawback to this approach is increased sample preparation.

A third technique for introducing a sample to the column is referred to as direct wet sample application. In this technique the sample is dissolved in a strong solvent and transferred to the head of the column. After this addition, the strong solvent is allowed to evaporate before the head of the column is reattached and the purification begun. Care must be taken to avoid disturbing the column bed when applying the sample.

Solid injection is another technique for difficult dissolution problems [14]. A dry, solid sample is ground to remove any large particles and increase the surface area. The ground material is mixed with stationary phase and the mixed matrix is packed into a sample column. The sample must be dry. Added safety precautions should be observed owing to increased sample handling.

With these techniques, preparative chromatographers can solve the sample solubility problems encountered when chlorinated solvents are eliminated. Fig. 6 shows the extent to which Searle chromatographers have used all of the above techniques.

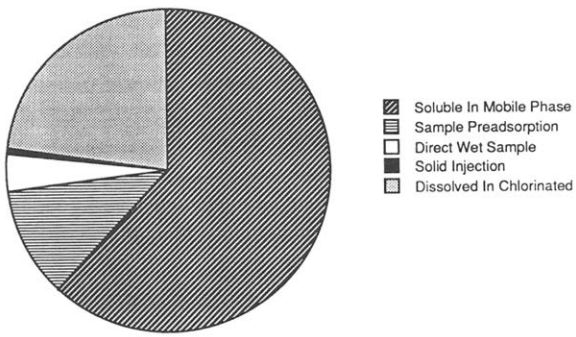

Fig. 6. Alternative sample application techniques employed by Searle preparative chromatographers. Data were collected from the purification of 684 samples (soluble in mobile phase = 417, dissolution in chlorinated solvent = 152, sample preadsorption = 75, direct wet sample = 24, solid injection = 4).

CONCLUSIONS

Over the last 3 years, the Preparative Chromatography Group has worked to reduce and eliminate the use of chlorinated solvents in liquid chromatography. Ethyl acetate and methyl *tert.*-butyl ether have been shown to be good substitutes for methylene chloride; nevertheless, a small number of purifications still require chlorinated solvents. In these instances a reduction technique is used to minimize the volume of chlorinated solvent. With these experiences, the group is now more efficient in eliminating chlorinated solvents, and will continue to investigate alternatives.

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