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# SOLID INJECTION, A NEW TECHNIQUE FOR APPLICATION OF INSOLUBLE SAMPLES IN PREPARATIVE LIQUID CHROMATOGRAPHY

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## SUMMARY

A new technique for the application of insoluble samples in preparative liquid chromatography has been developed. This technique, solid injection, greatly reduces the time and inefficiencies associated with techniques previously developed for this problem. Examples of purifications accomplished with this technique are given for compounds of pharmaceutical interest.

## INTRODUCTION

In preparative liquid chromatography one of the important steps in a purification is the application of the sample to the column. To obtain best results and to minimize potential problems the sample should be dissolved in a minimal volume of mobile phase[1]. Sometimes this is not possible due to extreme insolubility (< 10–100 mg/ml) of the sample in the mobile phase. Occasionally, this problem can be solved by dissolving the sample in a large volume of mobile phase, but this may reduce resolution and efficiency[2]. A stronger eluting solvent may also be used to increase solubility of the sample. This technique can be problematic and give undesired results[1]. Another way to apply insoluble samples to a preparative column involves pre-adsorbing the sample onto the stationary phase[3–6]. In this process, the sample is dissolved in any solvent, combined with stationary phase and the solvent evaporated producing a dry, powdered matrix of sample coated onto the stationary phase. The coated stationary phase is dry-packed into a column which is inserted in the preparative chromatography system prior to the main column. For this technique to work, it is important that all of the solvent is removed. A potential problem is the degradation of the sample during solvent removal. This process can give desirable results but is time consuming.

In this paper we will discuss a new technique we have developed for dealing with insoluble samples. This technique, which we have named solid injection, requires less time for sample preparation and eliminates any possibility of sample degradation during sample application. In addition, examples of purifications utilizing this technique will be described.

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## EXPERIMENTAL

### *Equipment and materials*

The large-scale preparative liquid chromatograph was either a Sep Tech Model ST/800A or ST/800C (Wakefield, RI, U.S.A.). The small-scale preparative liquid chromatography system was a modular system sold by Beckman (Berkeley, CA, U.S.A.). The preparative columns varied in size from 250 mm × 10 mm I.D. to 6 ft. × 6 in. I.D. and were obtained from a variety of sources. The bulk packings were ICN adsorbents 32–63 μm, 60 Å irregular silica gel from ICN Biomedicals (Cleveland, OH, U.S.A.), Merck silica gel 60, 40–63 μm, 60 Å irregular silica gel from EM Science (Cherry Hill, NJ, U.S.A.) or Partisil Prep 20 ODS-3 and Prep 40 ODS-3 from Whatman (Clifton, NJ, U.S.A.).

The analytical chromatograph consisted of a Waters Assoc. Model 590 solvent delivery system and a U6K injector or Waters Intelligent Sample Processor (Milford, MA, U.S.A.), a Kratos Model 783 variable-wavelength detector (Ramsey, NJ, U.S.A.), a Linear Model 585 recorder (Hackensack, NJ, U.S.A.), and a Digital Equipment Corporation VAX 11/785 computer with Searle chromatography data system.

All chemicals for purification were synthesized in the Chemical Development laboratories of G. D. Searle & Co. (Skokie, IL, U.S.A.). The solvents were reagent grade or better and obtained from a variety of sources.

### *Preparation of sample for solid injection*

To achieve optimum results it is important that the chemical being purified is crushed to a fine powder. This is done to avoid slow dissolution of large particles. The crushed sample is thoroughly mixed with packing material to ensure homogeneity. The mixture is dry-packed into a sample column, and any remaining space is filled with packing material. As an alternative, packing material from the top of the main column can be removed and replaced with the packing and sample mixture. This is especially useful with axial compression columns.

The amount of packing to be mixed with the crushed sample is dependent on the relative solubility of the sample. For extremely insoluble samples (< 5 mg/ml), between five and ten parts packing to one part sample is needed. This reduces the chance of crystallization of the chemical which could block the sample column. For samples with intermediate solubility (5 to 50 mg/ml), between two and five parts packing to one part sample should be sufficient.

## RESULTS AND DISCUSSION

Solid injection is a technique which was developed to deal with the insoluble samples encountered during the purification of potential drug products. These samples become especially difficult to dissolve at the preparative loadings (50–100 mg sample per gram packing) used for these purifications. This technique is especially useful with samples exhibiting solubility of less than 100 mg/ml in the mobile phase. Solid injection has been used successfully with samples having solubilities of less than 1 mg/ml. It can be used with either silica gel, bonded normal-phase or bonded reversed-phase packings.

The column containing the packing and sample mixture should be inserted into the preparative system in a vertical position. This is necessary since a void will form in the column as the sample dissolves and enters the main column. If the column is in a horizontal position, a channel will form and most of the sample will never be dissolved. Initially, a lower flow-rate can be used to allow for efficient dissolution of the sample, although good results have been obtained using either a low or normal flow-rate of mobile phase.

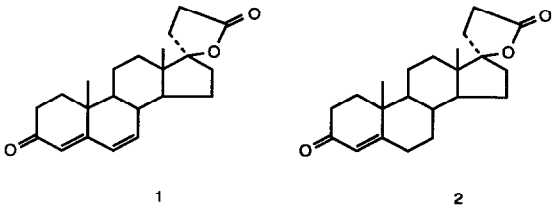
To ensure that highly retained compounds enter the main column, the sample column should be kept in the preparative system throughout the run. Column switching has also been utilized to selectively dissolve and separate portions of a sample. For example, when purifying a sample containing early eluting impurities, the eluent from the sample column can be diverted to a collection vessel while these impurities dissolve and exit the sample column. Once they have been removed from the sample column, the eluent can be diverted to the main column. Conversely, with highly retained impurities, after the desired component of the mixture has dissolved and entered the main column, the sample column can be removed from the solvent stream.

If air is detrimental to the adsorbent bed in the main column, one can pump a known amount of solvent through the sample column so that the packing is wetted before connecting it to the main column. This should serve to displace a large percentage of the air present.

*Examples*

*Purification of compound 1.* Compound 1 (Fig. 1) needed to be purified to >99.5% for preparation of a highly pure standard. The only impurity present was compound 2 (Fig. 1) at *ca.* 1%. The analytical high-performance liquid chromatographic (HPLC) separation of compounds 1 and 2 is shown in Fig. 2. This method was scaled up for preparative purification. Sample preparation involved mixing the sample (88 g) with 200 g of packing. The sample was purified on 4900 g of Partisil Prep 40 ODS-3 (53 μm) at a loading of 18 mg sample per gram of packing. A mobile phase of acetonitrile–water (40:60, v/v) and a flow-rate of 800 ml/min was used. The results of this purification are summarized in Table I. Using this method we were able to isolate 88% of the available compound 1 at a purity of >99.8%. The amount of compound 2 was reduced from 1.05% to 0.15% during the purification.

*Purification of compound 3.* There were two objectives to be met during the purification of compound 3 (Fig. 3): (1) production of chemical (>99%) for a highly pure standard, and (2) enrichment of unknown impurities for further isolation and



1 2

Fig. 1. Structure of compounds 1 and 2.

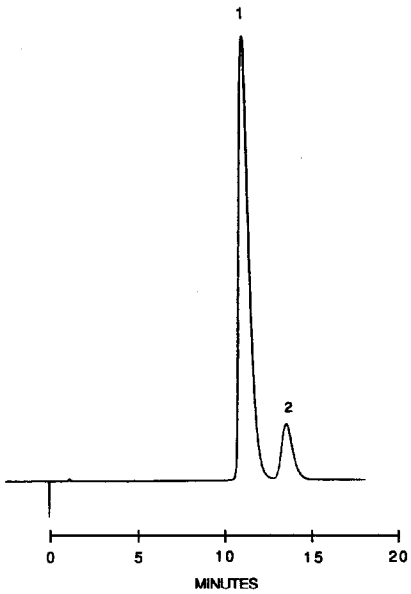



Fig. 2. Analytical HPLC separation of compounds 1 and 2. Analysis conducted on Partisil 10 ODS-3 (250 mm × 4.6 mm I.D.) with a mobile phase of acetonitrile–water (40:60). A flow-rate of 2 ml/min and detection at 254 nm, 0.1 a.u.f.s. was used. The sample shown was spiked with compound 2 for demonstration purposes and is not representative of the sample that was purified.

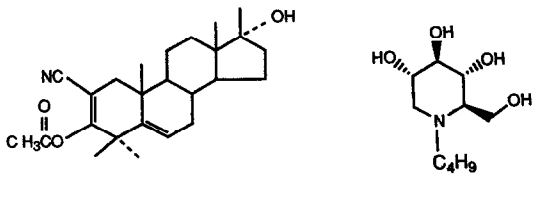
identification work. The analytical HPLC method and retention times of the desired components are summarized in Table II. Sample preparation for the preparative purification involved mixing the sample (94 g) with 700 g of Partisil Prep 40 ODS-3 (53 μm). The sample was purified on 4900 g of Partisil Prep 40 ODS-3 (53 μm) at a loading of 19 mg sample per gram of packing. A mobile phase of acetonitrile–water (50:50) and a flow-rate of 425 ml/min was used. The results of this purification are summarized in Table III. Although the main component eluted over a large volume, resulting in a long purification method, we were able to isolate 51% of the available compound 3 at a purity of >99.8%. In addition, another 23% of the chemical was

TABLE I

RESULTS OF PREPARATIVE PURIFICATION OF COMPOUND 1

| *Fraction* | *k' (start)*[a] | *k' (end)*[b] | *Weight (g)* | *Compound 2 (%)*[c] | *Compound 1 (%)*[c] |
|---|---|---|---|---|---|
| S[d] | – | – | 87.4 | 1.05 | 98.95 |
| A | 13 | 20 | 74.6 | 0.15 | 99.85 |
| B | 20 | 21 | 5.23 | 1.05 | 98.95 |
| C | 21 | 24 | 4.68 | 8.11 | 91.89 |

[a] Capacity factor for start of fraction.
[b] Capacity factor for end of fraction.
[c] HPLC area percent.
[d] Sample prior to purification.




3 4

Fig. 3. Structure of compounds 3 and 4.

produced at a purity slightly less than 99%. The three impurities were greatly concentrated in the fractions not containing compound 3. This made the subsequent isolation of each of these three impurities (also done using solid injection) much less time consuming.

*Purification of compound 4.* Compound 4 (Fig. 3) needed to be purified to >99% for preparation of a highly pure standard. The purification method was scaled up from the thin-layer chromatography method described in Fig. 4. Sample preparation involved mixing the sample (14.6 g) with 74 g of Merck silica gel (40–63 μm). The sample was purified on 1000 g of silica gel at a loading of 14 mg sample per gram of packing. A step gradient of varying percentages of chloroform, methanol and ammonium hydroxide and a flow-rate of 500 ml/min were used. Thin-layer chromatographic analysis of the individual fractions from this purification is shown in Fig. 4. Using this method we were able to isolate 67% of the available chemical at a purity of >99.8%.

TABLE II

ANALYTICAL HPLC DATA FOR COMPOUND 3

HPLC conditions: Partisil 5 ODS-3 RAC II (100 mm × 4.6 mm I.D.); mobile phase, acetonitrile–water (60:40) adjusted to pH 3.0 with phosphoric acid; 2.0 ml/min; detection, 238 nm, 0.1 a.u.f.s.

| *Component* | *Retention time (min)* | *HPLC area%* |
|---|---|---|
| Impurity 1 | 1.42 | 0.37 |
| Compound 3 | 3.68 | 97.51 |
| Impurity 2 | 4.85 | 0.36 |
| Impurity 4 | 5.23 | 0.09 |

TABLE III

RESULTS OF PREPARATIVE PURIFICATION OF COMPOUND 3

| *Fraction* | *k' (start)* | *k' (end)* | *Weight (g)* | *Purity (%)*[a] |
|---|---|---|---|---|
| S | – | – | 98.4 | 97.51 |
| A | 21 | 22.5 | 22.1 | 98.42 |
| B | 22.5 | 31.5 | 49.2 | 99.86 |

[a] HPLC area%.

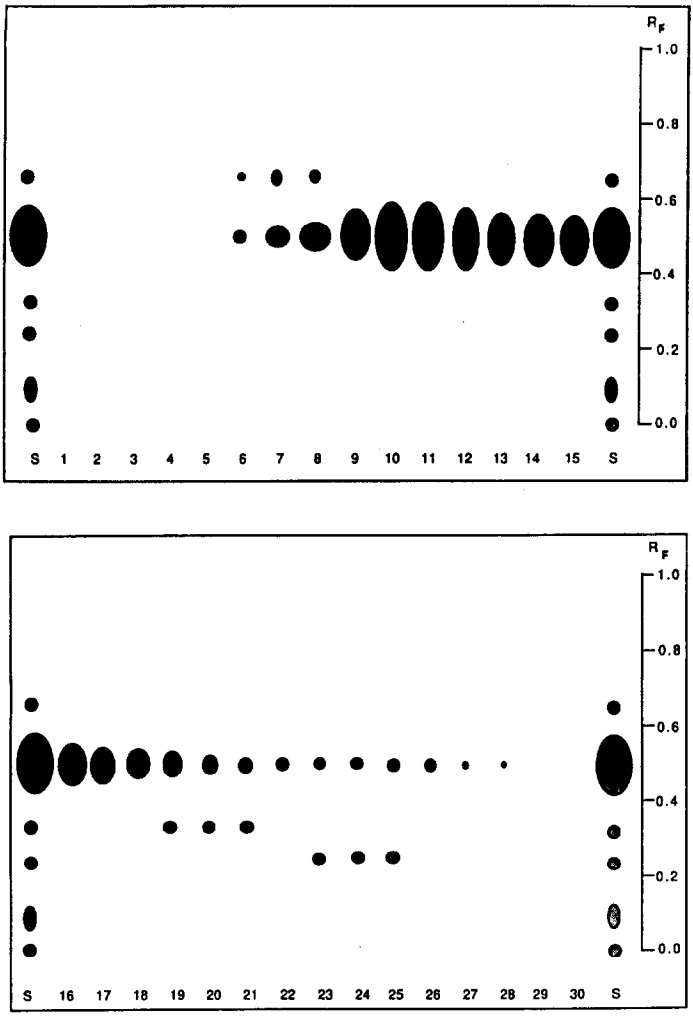



Fig. 4. Thin-layer chromatographic analysis of fractions from the preparative purification of compound 4. Solvent system, chloroform–methanol–ammonium hydroxide (40:58:2); detection, *tert.*-butyl hypochlorite–starch–potassium iodide; TLC adsorbent, Merck silica gel 60 $F_{254}$. S is sample prior to chromatography. Each fraction is one half of a column volume.

## CONCLUSION

Since the development of this technique approximately four years ago, we have proven it to be extremely versatile and time saving. Good results have been obtained on more than fifty purifications using both silica gel and reversed-phase packing materials. Our purification objectives have always been met whenever we have used this solid injection technique. The compound types which have been used with this technique include steroids, di- and tripeptides, fatty acid derivatives, sugars, amino

sugars, quinolones, heterocyclics and prostaglandin precursors. This technique has proven useful for samples ranging from 0.1 g to 1900 g in weight. The majority of this work was done with low-molecular-weight ($<600$) compounds. Solid injection may also be useful with other types of compounds.

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