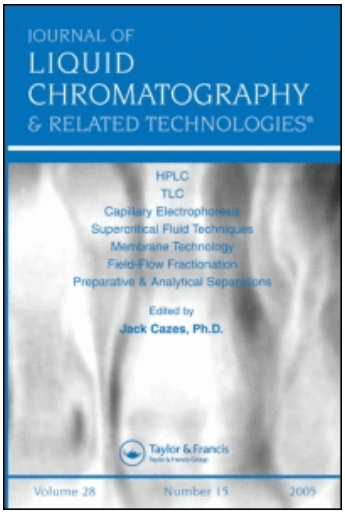

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Preparative Isolation of Pyrrolizidine Alkaloids Derived from Senecio Vulgaris

H. J. Segall[a]
[a] Department of Physiological Sciences, University of California School of Veterinary Medicine, Davis, California

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PREPARATIVE ISOLATION OF PYRROLIZIDINE ALKALOIDS DERIVED FROM SENECIO VULGARIS

H.J. Segall
University of California
School of Veterinary Medicine
Department of Physiological Sciences
Davis, California 95616

ABSTRACT

A large scale method for the isolation of pyrrolizidine alkaloids has been adapted from a previously published analytical method. This method is a reverse phase high pressure liquid chromatography technique, utilizing a .005 M KH_2PO_4 (pH 6.3) -- methanol solvent system. The pyrrolizidine alkaloids derived from Senecio vulgaris (common groundsel) have been utilized in this system.

INTRODUCTION

Senecio vulgaris (common groundsel) is found in many areas of the world, including Northern California[1]. Recently, an increase in both the pyrrolizidine containing plants (Senecio vulgaris, S. jacobaea, S. longilobus) and the number of livestock affected by these toxic plants[2] led us to develop a high pressure liquid chromatography (HPLC) isolation technique adaptable to a variety of Senecio species[3,4,5].

In a recent article, my laboratory determined a reverse phase HPLC procedure to isolate individual pyrrolizidine alkaloids[6]. That article discussed the feasibility of using reverse phase HPLC to isolate pyrrolizidine alkaloids from a variety of species. This article will discuss the feasibility of using preparative HPLC to isolate the 3 pyrrolizidine alkaloids (Retrorsine, Seneciphylline, Senecionine) from S. vulgaris.

MATERIALS AND METHODS

Senecio vulgaris was obtained from the Clark farm located near Turlock, California. The alkaloids were obtained by refluxing the ground dried plant material with methanol for at least 25 hours (Soxhlet). The methanol was removed under reduced pressure, the extract solubilized with 2N H_2SO_4, and filtered (Whatman #1). The acid aqueous phase was extracted with mixed hexanes and diethylether to insure removal of chlorophyll and waxes. Excess zinc dust was added to the acid aqueous phase to reduce any N-oxides of the pyrrolizidine alkaloids and stirred overnight at room temperature. The solution was filtered and the pH adjusted to 9 with ammonium hydroxide. The alkaline solution was extracted with chloroform, washed with water, dried over sodium sulfate and evaporated under reduced pressure. The pyrrolizidine alkaloids were dissolved in ethanol and filtered through celite to remove the yellowish color. They were then recrystallized from ethanol. A 500 mg mixture of pyrrolizidine alkaloids dissolved in 110 ml of methanol was the usual sample size.

A Waters Associate prep 500 system which has the capacity for two prep-500/C-18 reverse phase columns was utilized. This system includes a refractometer and recorder which were utilized to identify the pyrrolizidine alkaloids. Utilizing a flow rate of 150 ml/min., an isocratic gradient was run with a 60% methanol-40%/.005 M potassium phosphate (.005 M KH_2PO_4) buffer (pH 6.3).

All HPLC peaks were analyzed by gas chromatography - mass spectrometry. A 3% OV-17, 80/100, 4' long column with an oven temperature of 220°C, was used with an LKB-9000 mass spec plus computer hookup and printout (Digital PDP-8).

RESULTS

The following separation was achieved by using the mixed pyrrolizidine alkaloids of S. vulgaris. An isocratic HPLC condition of 60% Methanol - 40% .005 M KH_2PO_4 (pH 6.3) is illustrated in figure 1. The pyrrolizidine alkaloids Retrorsine, Seneciphylline and Senecionine eluted in the customary fashion, with Retrorsine eluting first (Peak# 1) followed by Seneciphylline (Peak #2), and Senecionine (Peak #3). My laboratory has found it feasible to collect the initial Retrorsine peak (Peak #1) and to recycle the Seneciphylline and Senecionine peaks. On the second pass through the 2 columns, the separation between Seneciphylline and Senecionine (Peaks 2 and 3) is sufficient to collect pure samples of both pyrrolizidine alkaloids (figure 1). When samples were not collected, the eluate was sent to waste to minimize any accidental contamination.

Samples from the prep 500 system were also checked on the Waters Analytical reverse phase column following the previously described procedure[6]. Figure 2 illustrates the purity of Senecionine which has a retention time of 15.5 min, using the analytical system[6].

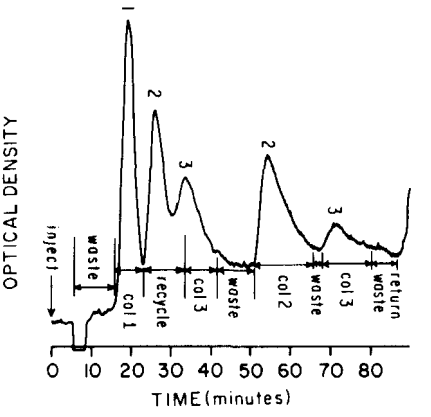

Figure 1 A preparative isocratic analysis of pyrrolizidine alkaloids derived from Senecio vulgaris. A 60% Methanol-40% .005 M KH_2PO_4 (pH 6.3) solvent system was used with two prep-500/C18 reverse phase columns plus flow rate of 150ml/min. The sample consists of 500 mg of pyrrolizidine alkaloids dissolved in 110 ml of methanol. Peak #1 = Retrorsine, Peak #2 = Seneciphylline, Peak #3 = Senecionine.

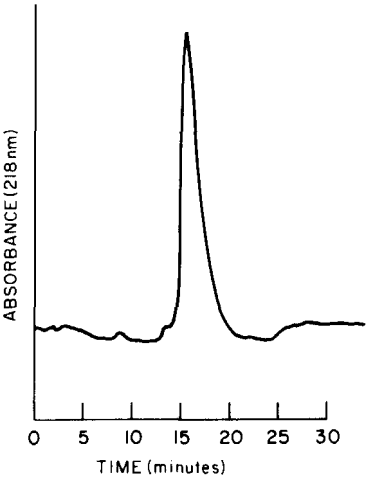

Figure 2 All peaks isolated from the preparative reverse phase system were checked on the analytical reverse system (Reference 6). The sample illustrated here is Senecionine which has a retention time of 15.5 minutes.

DISCUSSION

This paper has presented the first large scale HPLC separation of pyrrolizidine alkaloids derived from S. vulgaris. The pyrrolizidine alkaloids which have been isolated and purified are Retrorsine, Seneciphylline, and Senecionine.

An important advantage of using a reverse phase preparative system with 60% Methanol -40% .005 M KH_2PO_4 (pH 6.3) is that solvent costs may be kept at a minimum. The quantity of solvent used at 150ml/min. is quite high, even using the recycle mode. At this rate, the cost of most organic solvents would become prohibitive.

It is advisable to purify the mixture of pyrrolizidine alkaloids prior to loading them onto the HPLC to facilitate separation. The prep 500 HPLC system uses only a refractometer to identify various peaks which is not a refined system. There is a splitter in the line from which a UV or flourescent detector might be utilized to reconfirm the presence of pyrrolizidine alkaloids (218nm).

One cannot overemphasize the importance of recycling the Seneciphylline and Senecionine peaks (Figure 1, Peaks 2 and 3), as they are too close to achieve proper separation in the initial pass through the system. We do recommend that the last portion (40%) of the Senecionine (Peak 3) be collected on the initial pass through the system, as it will not be contaminated with Seneciphylline. The remaining 60% of the Senecionine (peak 3) should be collected on the second pass through the system. We have also used flow rates of 100ml/min and 200ml/min and found these flow rates to provide adequate separation. The flow rate of 150 ml/min is easily handled in my laboratory, and should not be considered the maximum flow rate which one may utilize in the isolation of pyrrolizidine alkaloids.

The prep-500/C18 reverse phase bondapak columns are subjected to splitting if constantly depressurized and repressurized when changing nitrogen tanks. Waters-Associates only provides a 2 way valve which does not allow the operator of the instrument to maintain constant pressure on the columns while changing nitrogen tanks. To obtain maximum column life, replace the 2 way ball valve with a 3 way ball valve (Whitney-42XF2), as pressure may be maintained on the columns while removing all pressure from the nitrogen regulator and tank.

The disadvantage of the preparative unit is that it is not engineered to run gradients. The operator must determine the optimum range of conditions on an analytical HPLC unit and then after a few trials determine the proper conditions for preparative HPLC. My laboratory is interested if our present prep system is applicable to pyrrolizidine alkaloids derived from other plants. The ability to apply this system to other pyrrolizidine alkaloids will allow the isolation and identification of many "new" pyrrolizidine alkaloids.

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