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Note

High-performance liquid chromatographic analysis of pyrrolizidine (*Senecio*) alkaloids using a reversed-phase styrene–divinylbenzene resin column

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Pyrrolizidine alkaloids (PAs) have attracted considerable attention due to their hepatotoxic effects[1] and occurrence in a large number of plant species[2]. PAs have caused significant losses of livestock[3] and have been implicated in human poisoning[4]. *Senecio jacobaea* (tansy ragwort) and *S. vulgaris* (common groundsel) are two common PA-containing plants occurring in the Pacific Northwest. Tansy ragwort is responsible for serious livestock losses in that area[5] and contamination of milk[6] and honey[7] by its alkaloids has been reported.

We have previously used[8] a C_8 reversed-phase high-performance liquid chromatographic (HPLC) system for the analysis of *Senecio* PAs. This method, as well as one using other reversed-phase columns[9,10], has certain drawbacks, however, such as shortened column life due to the relatively high pH of the buffers used, significant peak tailing with resulting limitations in resolution, limited sensitivity with gradient analysis because of solvent (methanol or tetrahydrofuran) absorption and pump seal wear from buffer salts. We report here a method using a reversed-phase styrene–divinylbenzene resin HPLC column that overcomes most of these problems and which may be ideally suited for use in quantitative analysis of the PAs.

MATERIALS AND METHODS

S. vulgaris plants and *S. jacobaea* flower tops were collected in the vicinity of Corvallis, OR, U.S.A., during May and July of 1979, respectively. Inorganic chemicals used were reagent grade (Mallinckrodt, St. Louis, MO, U.S.A.) and extraction solvents were glass distilled in our laboratory. The PAs jacobine, jacoline, senecionine and seneciphylline were isolated from recrystallized *S. jacobaea* extracts by preparative reversed-phase HPLC. The recovered PAs were recrystallized and their purity verified by analytical HPLC, melting point and gas chromatographic–mass spectrometric analysis.

Extracts were prepared from each plant species in the same manner, following the method used by Culvenor and co-workers[2,11] and others. The plant material was extracted with methanol in a Soxhlet apparatus for 16–24 h, the extract filtered and the solvent removed at reduced pressure. The residue was taken up in a mixture of *n*-

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hexane and 0.2 *N* sulfuric acid. The phases were separated and then the aqueous layer was extracted three times with *n*-hexane and four times with dichloromethane. The alkaloid N-oxides were reduced by adding zinc dust to the acidic aqueous solution and the mixture was stirred for 1 h. It was then filtered and the filtrate was extracted four times with dichloromethane. The pH of the aqueous phase was then adjusted to 10.5 with concentrated NH_4OH. The solution was then extracted three times with dichloromethane. The combined organic fractions were dried over anhydrous Na_2SO_4. After filtration, the solvent was removed at reduced pressure. The extracts were dried over NaOH *in vacuo*.

A PRP-1 reversed-phase resin column (15 cm × 4.1 mm) (Hamilton, Reno, NV, U.S.A.) was used for the separation of the PAs. HPLC was performed on an Altex Model 322 chromatograph equipped with a Schoeffel SF-770 detector set at 220 nm. The chromatograms were recorded with a Spectra-Physics 4100 integrator.

The mobile phase consisted of acetonitrile (HPLC grade; J. T. Baker, Phillipsburg, NJ, U.S.A.) and 0.1 *M* NH_4OH made up using water purified with a Milli-Q system (Millipore, Bedford, MA, U.S.A.). Loss of NH_3 from this solution via volatilization did not cause any appreciable variation in results if the NH_4OH solution was used within two days of its preparation. *S. jacobaea* alkaloids were separated with a 20-min linear gradient of 10% to 30% acetonitrile. Separation of *S. vulgaris* alkaloids was achieved isocratically with 25% acetonitrile. A flow-rate of 1 ml/min was used in both cases.

Samples were dissolved in methanol–water (1:1) for injection. Peaks were collected and the solvents removed under vacuum. Direct probe electron impact mass spectra were obtained on a Varian CH-7 spectrometer coupled with a Systems Industries 150 computer.

RESULTS

Fig. 1 shows the separation of a mixture of pure jacoline, jacobine, seneciphylline and senecionine chromatographed with a 10 to 30% acetonitrile–NH_4OH gradient. The peaks are essentially symmetrical and retention times correspond to those of peaks in the chromatogram of a *S. jacobaea* extract (Fig. 2). Mass spectrometry of collected fractions from the crude *S. jacobaea* extract yielded the following assignments by comparison with published PA spectra and fragmentation patterns[2,7,10,12]: peak 3 = jacoline, peak 8 = jacozine, peak 9 = jacobine, peak 10 = jaconine, peak 11 = seneciphylline and peak 12 = senecionine.

Peaks 1, 2, 4 and 7 do not appear to be PAs. Peaks 5 and 6 gave spectra containing fragments characteristic of *Senecio* PAs but these compounds have not yet been identified.

Fig. 3 shows a chromatogram of a *S. vulgaris* extract obtained with the isocratic system. As with the gradient system, the peak shape is good. The major peaks were identified by comparison of the mass spectra with those available in the literature[2,7,12]: peak 1 = retrorsine, peak 2 = seneciphylline, and peak 3 = senecionine.

Fig. 4 shows detector response curves for jacoline, jacobine, senecionine and seneciphylline. Response (in area units) is linear over a 40-fold range of PA injected.

Three 1.00-g samples of dried *S. jacobaea* flowers were extracted and analyzed for jacoline, jacobine, seneciphylline and senecionine content using the 10–30% ace-

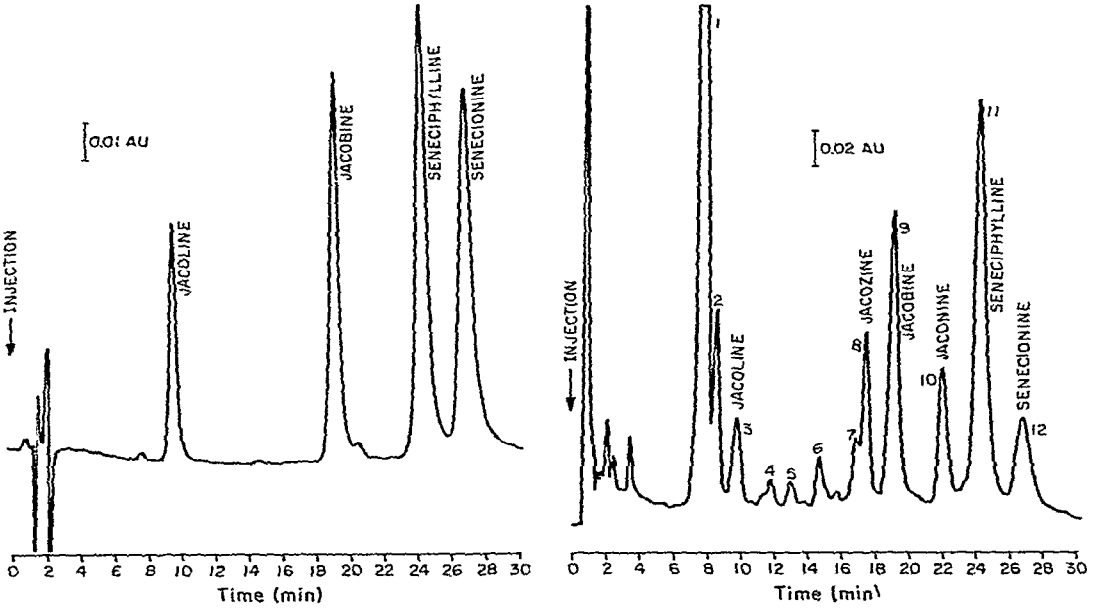

Fig. 1. Separation of a mixture of pure PAs. Solvent: 1 ml/min acetonitrile (10% to 30% in 20 min)–0.1 *M* NH_4OH.

Fig. 2. Separation of a *S. jacobaea* extract. Conditions as in Fig. 1.

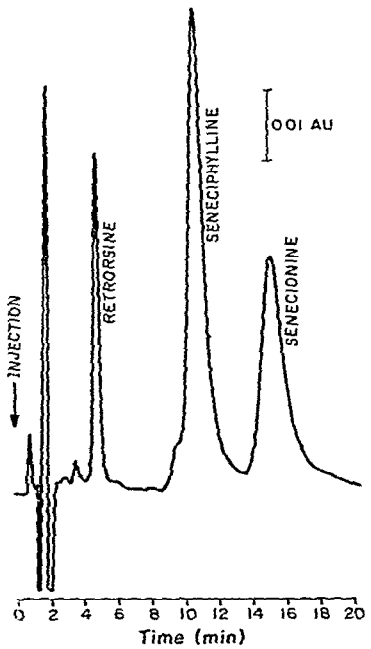

Fig. 3. Separation of a *S. vulgaris* extract. Solvent: isocratic, 1 ml/min, acetonitrile–0.1 *M* NH_4OH (25:75).

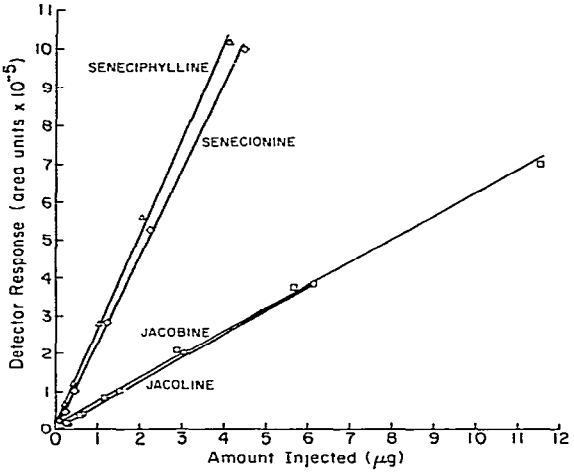

Fig. 4. Detector response *vs.* amount injected. Conditions as in Fig. 1. Each data point represents a single determination.

tonitrile gradient system. Average values for these PAs in μg/g dry weight ($\pm$ standard deviation) were, respectively, 155 ($\pm$ 17), 1019 ($\pm$ 35), 405 ($\pm$ 20) and 148 ($\pm$ 12). Total PA concentrations in dried *S. jacobaea* flowers have been previously reported to be 0.15–0.30%[7] and 0.2%[13].

DISCUSSION

A styrene–divinylbenzene resin column allows use of high pH mobile phases that would destroy silica-based columns. One result is good peak symmetry for basic compounds without the use of ion-pairing reagents. The acetonitrile–NH_4OH system offers several advantages. Preparative work is simplified since the mobile phase solvents can be directly evaporated under vacuum, thus avoiding the extractions necessary when using phosphate buffers. The low absorbance of HPLC-grade acetonitrile at 220 nm allows gradient analysis of PAs and high sensitivity without prohibitive baseline shifts. The problems associated with the use of solid buffer salts (including increased pump seal wear) are avoided with this system.

The method presented here will be useful for analysis of small samples of *S. jacobaea* and *S. vulgaris*. It should prove useful for analysis of other PA-containing extracts as well.

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* *Editor's Note:* See also H. J. Huizing and Th. M. Malingré, *J. Chromatogr.*, 176 (1979) 274.