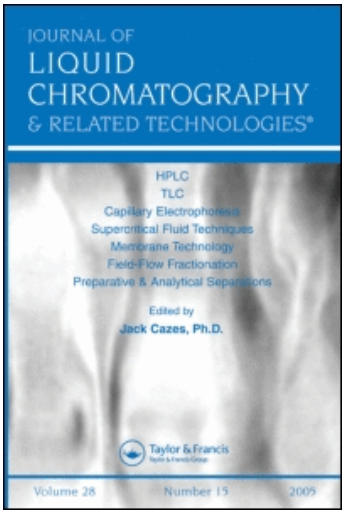

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Selection of a Suitable Solvent System for the Isolation of Toxicologically Active Components of an African Arrow Poison of Plant Origin

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To cite this Article Dennis, Michael and Ochillo, Richard F.(1981) 'Selection of a Suitable Solvent System for the Isolation of Toxicologically Active Components of an African Arrow Poison of Plant Origin', Journal of Liquid Chromatography & Related Technologies, 4: 10, 1847 — 1854
To link to this Article: DOI: 10.1080/01483918108064851
URL: http://dx.doi.org/10.1080/01483918108064851

JOURNAL OF LIQUID CHROMATOGRAPHY, 4(10), 1847-1854 (1981)

SELECTION OF A SUITABLE SOLVENT SYSTEM FOR THE ISOLATION OF TOXICOLOGICALLY ACTIVE COMPONENTS OF AN AFRICAN ARROW POISON OF PLANT ORIGIN

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ABSTRACT

The efficiency of different solvent systems in the isolation of toxicologically active components of an african arrow poison of plant origin was investigated. The solvent systems were: de-ionized water; acetonitrile; acetonitrile/water (9:1); gradient elution with carbon tetrachloride/chloroform (10-50%); gradient elution with sodium chloride (0.02M) acetonitrile (45-95%) and; methanol/de-ionized water (98:2) and; chloroform and carbon tetrachloride (10-50%) gradient elution. The gradient elution with sodium chloride (0.02M)/ acetonitrile (45-95%) gave the best separation of four distinct peaks suggesting the presence of at least four components in the arrow poison. De-ionized water eluted the arrow poison from the column in one single fraction, whose peak height was linearly related to the amount of toxin (0.02 - 20.0 μg) suggesting that HPLC might also be useful in quantifying the toxin and/or its active components.

INTRODUCTION

Drug development has become very expensive and the need for exploring new and relatively cheaper approaches for developing drugs has become urgent as the debates for medicare cost containment reaches a new crescendo. Traditional african medi-

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* Michael Dennis was a junior undergraduate when this investigation was carried out.

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cine is one of the possible new hopes and frontiers. African folk medicine provides a challenge for the development of new therapeutic agents based on phytotherapy, which has evolved over a long period of time. Accordingly, we have decided to toxicologically characterize an african arrow poison of plant origin by using modern chromatographic techniques to isolate the active component(s) and eventually investigate the mechanism of action of the toxin.

We previously used thin layer and ion exchange chromatographic methods to isolate the active components of the toxin (Cook, Dennis and Ochillo, 1979; Dennis, Cook and Ochillo, 1979; Cook, Dennis, Rolfs, Pugh and Ochillo, 1980; Cook, Dennis and Ochillo, 1981). Although these chromatographic techniques led to satisfactory separation of the active principles of the toxin, they are quite time-consuming and not readily subject to automatic methods of analysis. Therefore, we have used high performance liquid chromatography (HPLC) to separate toxicologically active components of the arrow poison and the results of our separation are reported in this manuscript. Also, we have attempted to use HPLC to quantify the toxin.

MATERIALS AND METHODS

Arrow poison is heat-extracted from plants by the natives of Kenya from where the sample used in this investigation was obtained. The procedures for preparation and the types of plants the poison is extracted from is in preparation and will be published elsewhere. However, the material under test was a dry extract. The chemical reagents used in this investigation were purchased from Sigma Chemical Company (St. Louis, MO) or from Burdick and Jackson Laboratories, (Muskegon, MI).

The acetonitrile, methanol, chloroform and carbon tetrachloride were used to elute the components of the toxin from the column as received from commercial sources. The sodium chloride solution was made from crystals as required. Appropriate elution solvent mixtures were prepared as follows: sodium chloride (0.02M)/acetonitrile (8:2); acetonitrile/deionized water (9:1) and; gradient elution of sodium chloride (0.02M)/acetoni-rile (45-95%), methanol/deionized water (98:2) and; chloroform and carbon tetrachloride (10-50%) gradient elution.

A 200mg portion of the poison (RFO-KMM-#1) was weighed and dissolved in de-ionized water and filtered through a millipore filter (pore size, 0.45 um). A 20 ul aliquot of the sample was injected into a Perkin-Elmer Series 3 HPL (Perkins-Elmer, Norwalk, Connecticut) equipped with a deuterium power supply, a digital programmer, an optical unit for detection of UV absorption at 260nm, a stainless steel HPLC column (25 x 0.26 cm) packed with Silica-A (HC-ODS-Sil-S, Lot 34) and a model 023 recorder. The flow rate was 1.0ml/minute (-cm/min.) with a pressure drop of 1800 psig at ambient temperature.

RESULTS AND DISCUSSION

The results of the separation of active principle(s) using HPLC and different solvent systems are presented in Figure 1-4. Since the separation using de-ionized water as a solvent led to the elution of only one peak, we decided to use this solvent to investigate the relation between the sample size injected into the column and the peak height. The relationship is shown in Fig. 5.

The results of our preliminary investigation indicated that the toxin is soluble in water, which is polar, suggest-

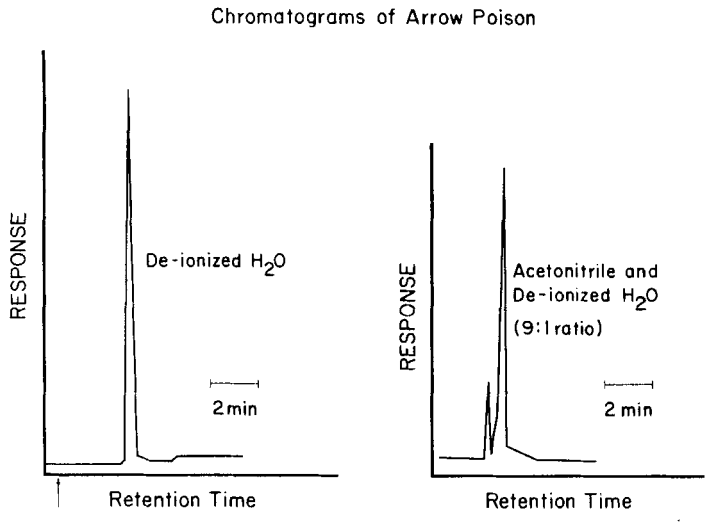

FIGURE 1.Chromatograms of eluted peaks of the arrow poison as a function of retention time. The solvent used for elution are shown in the figure.

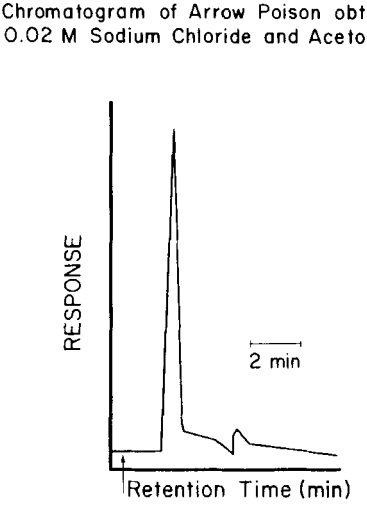

FIGURE 2.Elution pattern of arrow poison using sodium chloride and acetonitrile (8:2).

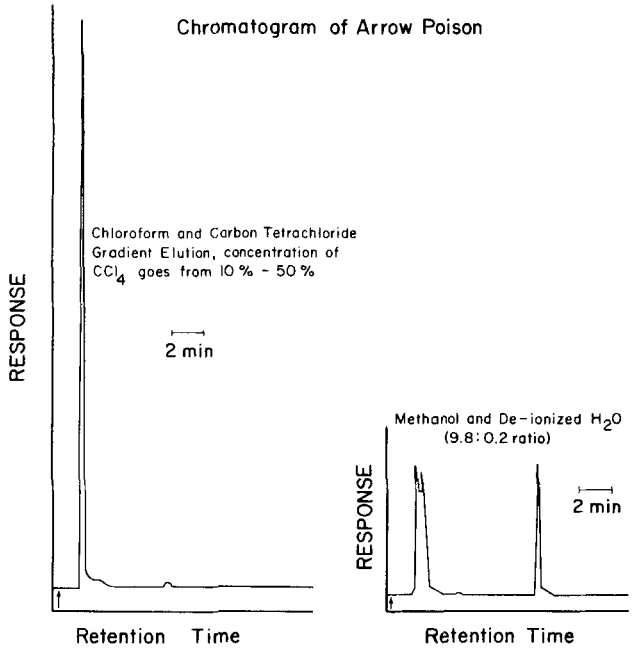

FIGURE 3. Elution profile using gradient elution of chloroform and carbon tetrachloride (10-50%) and; methanol and deionized water.

ing that the toxicologically active components therein are polar (Cooke et al. 1981.) Therefore, in our search for more suitable solvents for the separation of the active principles, we started with de-ionized water and varied the polarity of the solvent. This was accomplished by using different solvents and their mixtures such that polarity is changed until the appropriate solvent was identified. The best separation was achieved using gradient elution with 0.05M sodium chloride/ acetonitrile (45-95%) leading to a resolution of four distinct peaks (Fig. 4). In order of decreasing efficiency, the quality of separation achieved with the different solvents as shown in Figs. 1-4 can be arranged in the following order: gradient

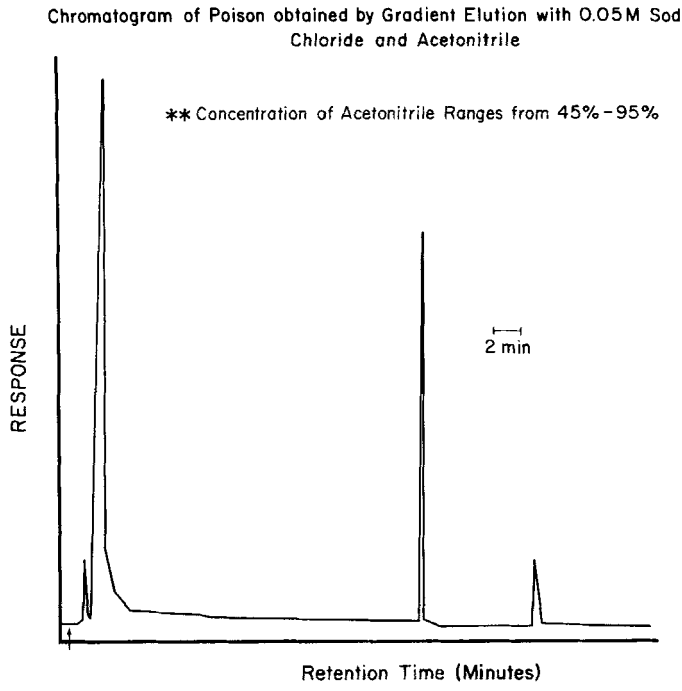

FIGURE 4.Elution profile using gradient elution with 0.05M sodium chloride and acetonitrile ranging in concentration from 45% to 95% (v/v).

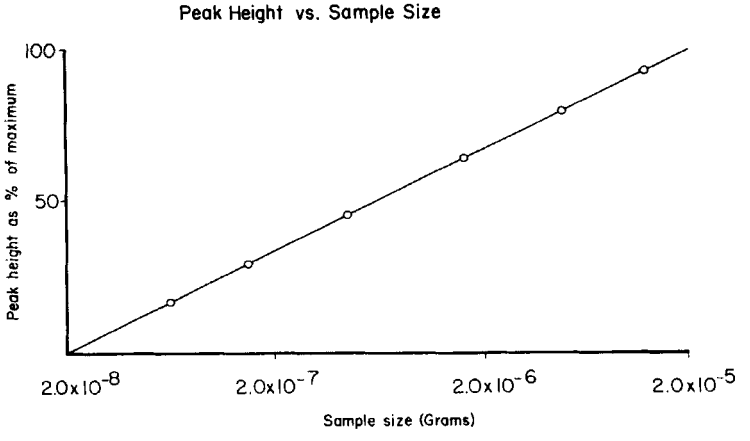

FIGURE 5.Calibration curve for solution of the arrow poison.

elution with 0.05 sodium chloride/acetonitrile (45-95%) > methanol and de-ionized water (98:2) > acetonitrile and de-ionized water 9:1) > gradient elution of chloroform/carbon tetrachloride (10-50%) > sodium chloride (0.02M) and acetonitrile (8:2) > de-ionized.

The relationship between the sample size and the peak height was linear for the toxin size ranging from 20ng to 20 μg (Fig. 5). This observation would provide evidence in support of the use of HPLC as a powerful tool for the separation and quantifying the arrow poison and its toxicologically active components. Currently, we are in the process of developing a sensitive bioassay within the same sensitivity range as the separated HPLC components; thus we can compare the two methods before deciding on which one might be most appropriate for investigating activities of the toxin.

ACKNOWLEDGEMENTS

This investigation was partially supported by Grant 2S06RR0800810 from the Division of Research Resources of the National Institute of Health, Bethesda, MD. Part of this work was presented at the Society of Pharmacology and Experimental Therapeutics Fall Meeting in Portland Oregon in August 1979. We thank Ms. Joan Milton for typing the manuscript.

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