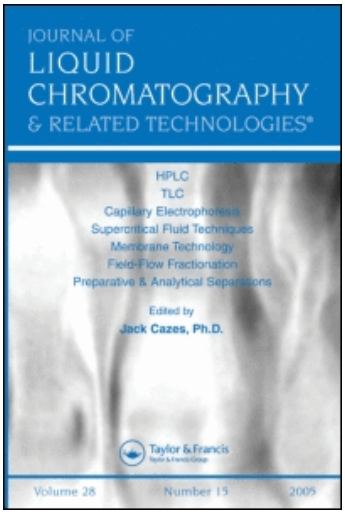

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ANALYSIS OF BIOLOGICAL TISSUES FOR SELENIUM BY THIN LAYER CHROMATOGRAPHY

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ABSTRACT

Selenium is oxidized by the method of Poole[1] as modified by Stahr, et al[4] to free it from matrix encumbrances. It is reduced by HCl dissolution and complexed with DAN (Diaminonapthalene) then extracted into cyclohexane and analyzed by TLC (reverse phase Whatman high performance plates) thin layer chromatography densitometry. TLC allows observation of 0.1 ng of the selenium (piazselenole) complex. Separation of the Se containing complex allows elimination of other potentially interfering fluorescent compounds. 100% recoveries of Se^{75} (radiolabelled) are achieved through the digest step and 85-90% are achieved through the complete test. Radioautography shows only the gold piazselenole spot contains selenium. Agreement with Bureau of Standards results on freeze dried liver is 100% $\pm$ 2%.

INTRODUCTION

Analysis for selenium is of great importance for health concerns. In the last decade it has been recognized as an essential element. Levels of significance for animals are presented in table one. Water analysis for selenium is done by hydrogen diffusion flame,[1] tantalum boats,[3] furnace atomic absorption[2] or by fluorometry.[2] Biological samples are more difficult to analyze due to matrix interferences. To do these samples, preparation must be done by digestion. The resulting

TABLE 1

Selected Selenium Values for Animal Tissues (ug/gm)

		DEFICIENT	MARGINAL	ADEQUATE
EQUINE	Serum	_ .050	.051 - .139	.140 - 0.300
	Liver	_ .160	.161 - .299	.300 - 1.000
BOVINE	Serum	_ .020	.021 - .069	.070 - 0.300
	Liver	_ .120	.121 - .249	.250 - 0.500
PORCINE	Serum	_ .060	.061 - .119	.120 - 0.300
	Liver	_ .120	.121 - .299	.300 - 0.800
OVINE	Serum	_ .030	.031 - .079	.080 - 0.500
	Liver	_ .150	.151 - .249	.250 - 1.000

solution may be analyzed by the above techniques, but, with difficulty, and often only by standard additions due to interferences.

The sample preparation procedure involves using Poole's[1] digest procedure, reduction, chelation, as modified by the Veterinary Diagnostic Laboratory. After partition into nonpolar solvent (cyclohexane) concentration and chromatography are done. Reverse phase thin layer chromatography analysis will be described here.

MATERIALS

Kontes Scanner 800, Vineland, NJ; Gamma Spectrometer (Chicago Nuclear, Chicago, IL); hydrochloric acid, ammonium hydroxide and nitric acid (Fisher Scientific Co., Itasca, IL); Millipore purified water, (Millipore Corp., Milford, MA); Spectrofluorometer, (Aminco Bowman, Silver Springs, MD); C_{18}

Reverse Phase TLC plates, (Whatman, Inc., Clifton, NJ); Silica gel TLC plates, E. Merck, (Brinkmann Instruments, Des Plaines, IL); Silica gel TLC plates, (Analtech, Wilmington, DE); freeze dried liver, (Bureau of Standards), fluorometer cells, (Fisher Scientific); Ultraviolet light - long wave length, (UV products, San Gabriel California); Fisher Recorder; Micropipets,(Clay Adams Division, Becton Dickinson, Research Triangle Park, NC); Diaminonapthalene, (Aldrich Chem Co., Milwaukee, WI); Cyclohexane, (Fisher Scientific); NaEDTA, (Baker Chemical Co., Phillipsburg, NJ); Hotplate, (Corning Company, Corning, NJ); Muffle Furnace, (Lindbergh Corp., Watertown, WI); Turner filter fluorometer, (Turner & Associates, Palo Alto, CA).

METHODS

Standards were added to liver samples and selenium was determined by the method of Poole[1] as modified by Vet. Diag. Lab.[2] Standard addition analyses were made by thin layer chromatography (TLC). 1/4 (volume/volume) ethyl acetate/toluene was used for normal phase TLC and 65/35/1, ethanol/water/acetic acid was used for reverse phase TLC. Fluorometric analysis was made on the same samples by filter fluorometer and spectrofluorometry. Bureau of Standards freeze dried liver was analyzed by the same techniques. Glucose was added to sample digests to determine if improved recoveries could be obtained.

Radioactive selenium was analyzed by these procedures and recoveries were determined at each step of the analysis. Radioautography was done on TLC separations to determine which bands contained radioactive selenium.

RESULTS

Figure one shows the typical appearance of selenium complex on TLC.

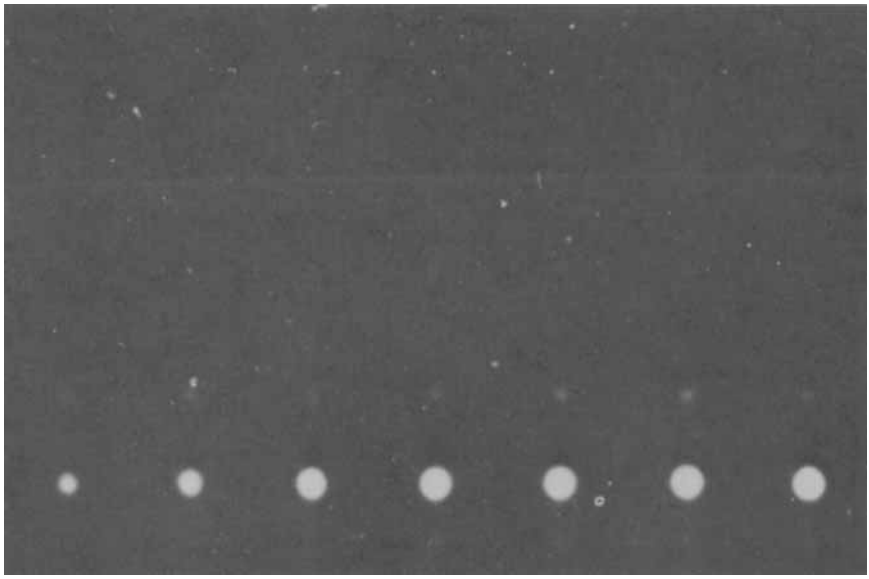


FIGURE 1
TLC Plate with 0.1-10 Nanograms of Selenium Complex

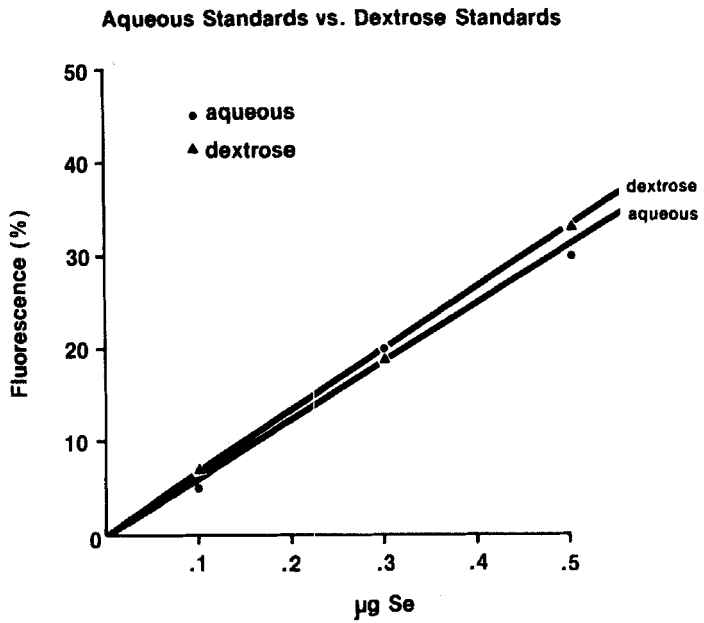
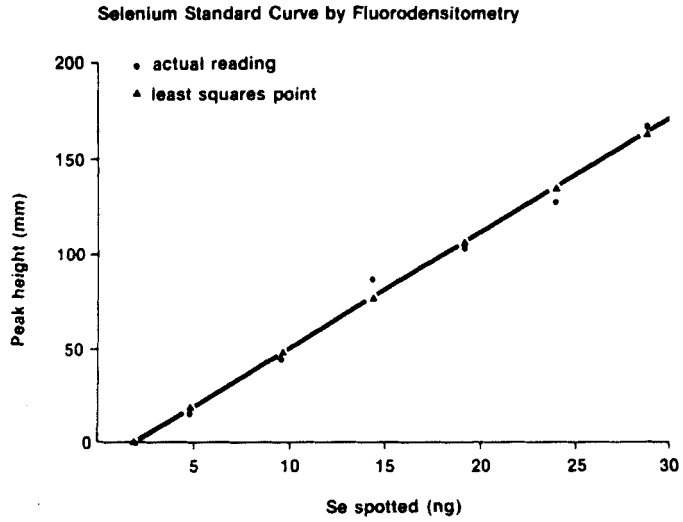


FIGURE 2
Fluorometric Analysis for Se Complex



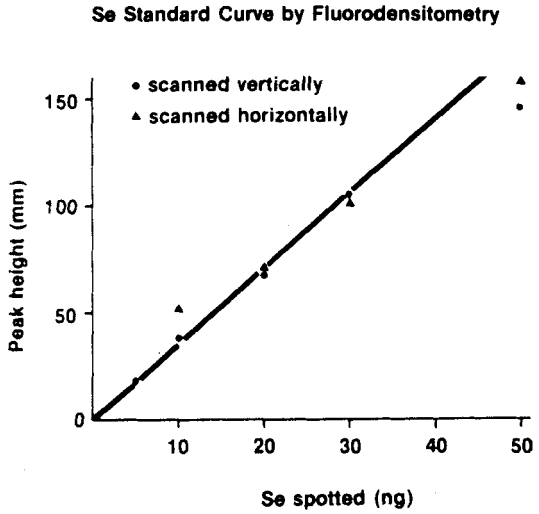


FIGURE 3
Densitometric Analysis for Se Complex

As little as 0.1 ng selenium is visible by TLC analysis. The effect of glucose on the analysis of selenium by fluorometric analysis is shown in figure two. Figure three shows two different studies of analysis of selenium by fluorodensitometry. A standard addition analysis by fluorometry and densitometry are shown in figure four.

Bureau of standards liver was found to contain 1.08 ppm - 1.10 ppm selenium by three different operators compared to a certified value of 1.10 (NBS) certified value. Figure five shows some of the bands on TLC which contain no selenium but which interfere with filter (wide band pass) fluorometeric analysis. The radioautograph showed that only one band contained selenium, the gold fluorescent band. 100% of the selenium was recovered through the ashing step in the procedure. An average of 90% of the radioactivity was recovered from the chelation partition steps. No further loss was observed on concentration for chromatography analysis.

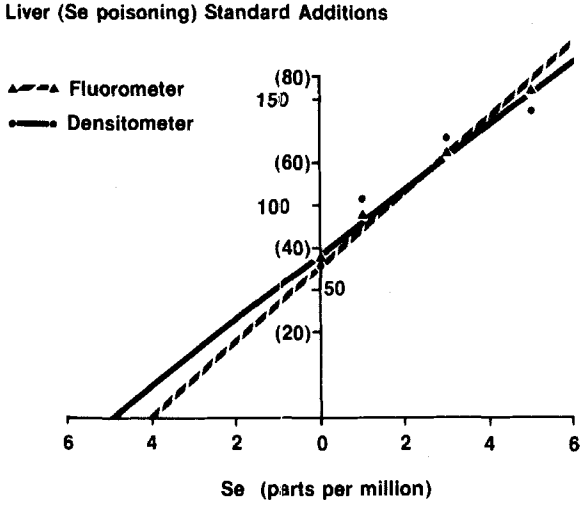


FIGURE 4
Standard Additions Analysis for Se by Two Methods

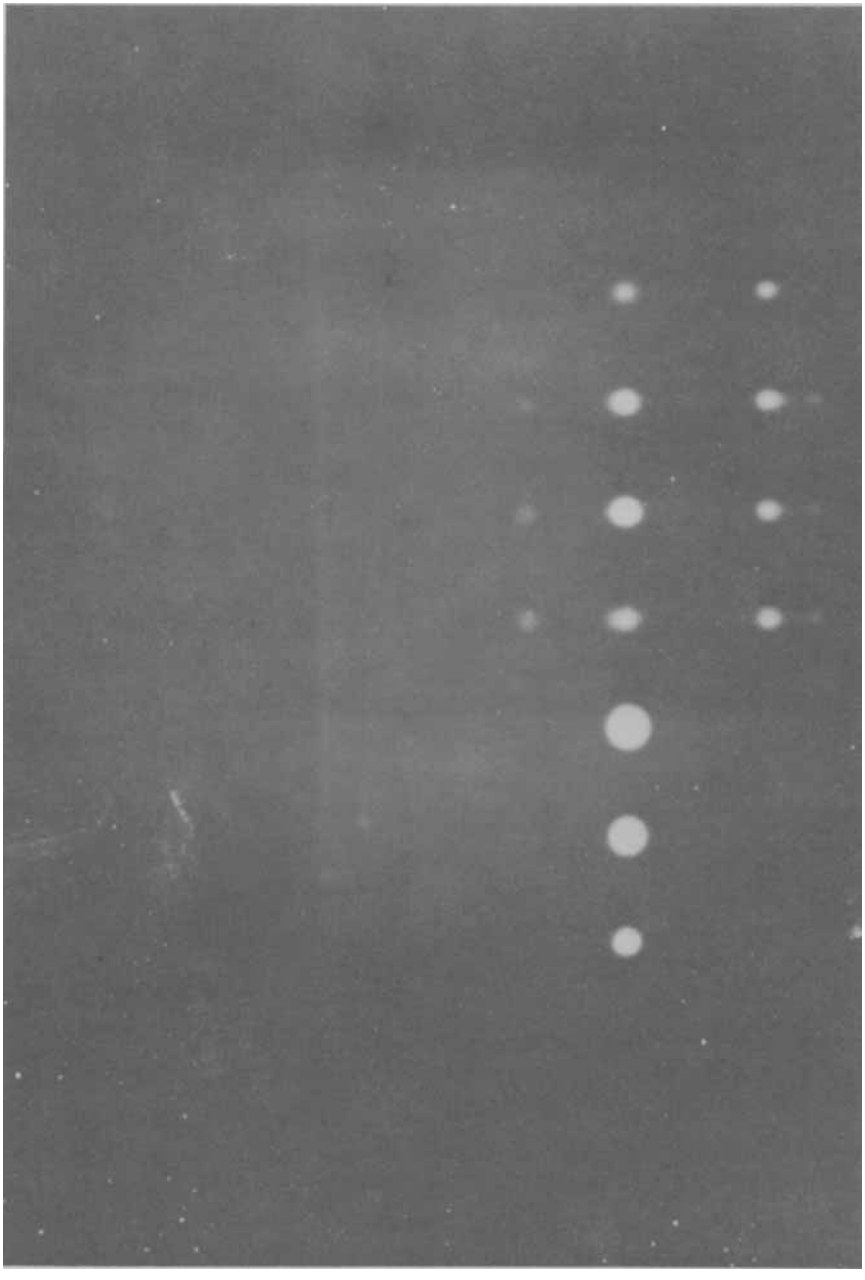


FIGURE 5
Fluorescent Bands from Tissue Analysis for Selenium

DISCUSSION AND CONCLUSIONS

It is concluded that direct thin layer chromatographic analysis for selenium may be done. The TLC detection limit is less than 0.1 ng. Reverse phase thin layer plates and normal phase TLC plates with selenium standards are shown in figure six. The reverse phase plates provide stable bands for days relative to hours for normal phase TLC plates. With simple visual detection (eyeball) techniques, selenium can be detected visually to 0.1 ng Using a Kontes 800 TLC scanner, it can be analyzed to nanogram levels $\pm$ 10%. Atomic absorption is extensively used for selenium analysis. Boat or cup analysis for selenium has been done for some time. Sensitivity is good (nanograms) but memory of previous samples provides a great effect.[5]

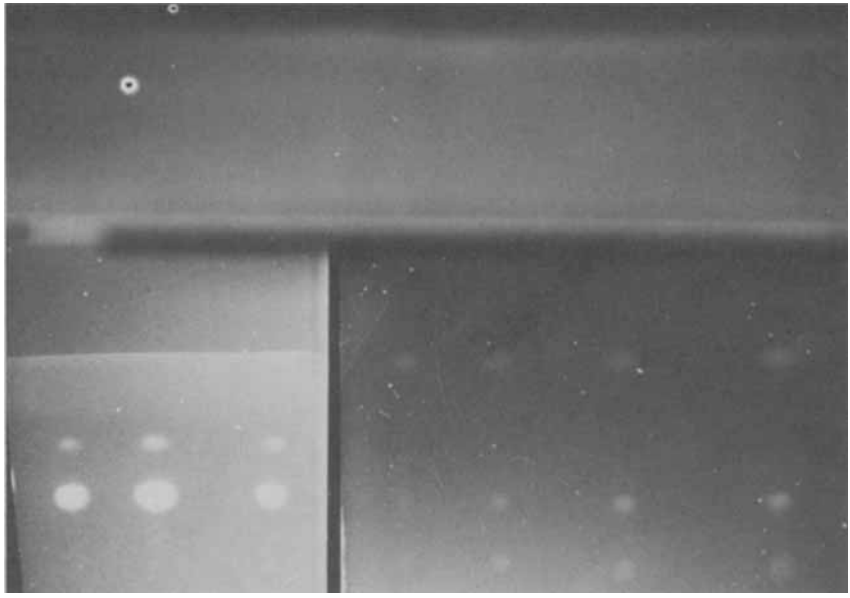


FIGURE 6
Selenium Complex on Normal and Reverse Phase TLC after 72 Hours

Flame analysis for selenium is hampered by scatter and background interference at the analytical line necessary for selenium analysis. One unit, the Hitachi, has a Zeeman background correction system which has been used in the application laboratory to determine selenium in the parts per million range. Without this system tens of parts per million are required to analyze for selenium [6].

The hydrogen diffusion flame or quartz tube (flame) with hydride generation suffer from complexation of selenium[7] requiring standard addition to correct for matrix effects.

The same effects make[7] nonflame furnace atomic absorption require standard additions. These latter two methods are sensitive to nanogram levels of selenium.

Thin layer chromatography with a densitometer is as sensitive as atomic absorption analysis and the chromatography provides a greater element of specificity.

The non-instrumental technique would be applicable to noninstrumental laboratories in the developing world. Direct TLC analysis saves time and TLC plates provide direct analysis of biological samples for selenium suitable for even trace analysis of deficient biological specimens.

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6. Unpublished data from Hitachi, Application Laboratory Courtesy of Michael Kamerlink, M.K. Associates, Chicago, Illinois.

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