UNIVERSITY OF CALIFORNIA — BERKELEY

RADIATION LABORATORY

DISCLAIMER

UCRL-824
Unclassified Distribution

UNIVERSITY OF CALIFORNIA

Radiation Laboratory

Contract No. W-7405-eng-48

PAPER CHROMATOGRAPHY OF STEROIDS

David Kritchevsky and M.Calvin

July 28, 1950

Berkeley, California

INSTALLATION:	No. of Copies
Argonne National Laboratory	8
Armed Forces Special Weapons Project	1
Atomic Energy Commission - Washington	2
Battelle Memorial Institute	1
Brush Beryllium Company	1
Brookhaven National Laboratory	4
Bureau of Medicine and Surgery	1
Bureau of Ships	1
Carbide and Carbon Chemicals Division (K-25 Plant)	4
Carbide and Carbon Chemicals Division (Y-12 Plant)	4
Chicago Operations Office	1
Columbia University (J. R. Dunning)	1
Columbia University (G. Failla)	1
Dow Chemical Company	1
H. K. Ferguson Company	1
General Electric, Richland	3
Harshaw Chemical Corporation	1
Idaho Operations Office	1
Iowa State College	2
Kansas City Operations Branch	1
Kellex Corporation	2
Knolls Atomic Power Laboratory	4
Los Alamos Scientific Laboratory	3
Mallinckrodt Chemical Works	1
Massachusetts Institute of Technology (A. Gaudin)	1
Massachusetts Institute of Technology (A. R. Kaufmann)	1
Mound Laboratory	3
National Advisory Committee for Aeronautics	1
National Bureau of Standards	3
Naval Radiological Defense Laboratory	2
New Brunswick Laboratory	1
New York Operations Office	3
North American Aviation, Inc.	1
Oak Ridge National Laboratory	8
Patent Branch (Washington)	1
Rand Corporation	1
Sandia Corporation	1
Santa Fe Operations Office	2
Sylvania Electric Products, Inc.	1
Technical Information Division (Oak Ridge)	15
USAF, Air Surgeon (Lt. Col. R. H. Blount)	1
USAF, Director of Armament (Captain C. I. Browne)	1
USAF, Director of Research and Development (Col. R. J. Mason, Fred W. Bruner)	2
USAF, Eglin Air Force Base (Major A. C. Field)	1
USAF, Kirtland Air Force Base (Col. Marcus F. Cooper)	1
USAF, Maxwell Air Force Base (Col. F. N. Moyers)	1
USAF, NEPA Office	2
USAF, Office of Atomic Energy (Col.H.C. Donelly, A.A. Fickel)	2
USAF, Offutt Air Force Base (Col. H. R. Sullivan, Jr.)	1
USAF, Wright-Patterson Air Force Base (Rodney Nudenberg)	1

INSTALLATION	No. of Copies
U. S. Army, Atomic Energy Branch (Lt. Col. A. W. Betts)	1
U. S. Army, Army Field Forces (Captain James Kerr)	1
U. S. Army, Commanding General, Chemical Corps Technical Command (Col. John A. MacLaughlin thru Mrs. Georgia S. Benjamin)	1
U. S. Army, Chief of Ordnance (Lt. Col. A. R. Del Campo)	1
U. S. Army, Commanding Officer, Watertown Arsenal (Col. Carroll H. Deitrick)	1
U. S. Army, Director of Operations Research (Dr. Ellis Johnson)	1
U. S. Army, Office of Engineers (Allen O'Leary)	1
U. S. Army, Office of the Chief Signal Officer (Curtis T. Clayton thru Maj. George C. Hunt)	1
U. S. Army, Office of the Surgeon General (Col. W. S. Stone)	1
U. S. Geological Survey (T. B. Nolan)	2
USAF, Director of Plans and Operations (Col. R.L. Applegate)	1
U. S. Public Health Service	1
University of California at Los Angeles	1
University of California Radiation Laboratory	5
University of Rochester	2
University of Washington	1
Western Reserve University	2
Westinghouse Electric Company	4
Naval Medical Research Institute	1
* University of Rochester not mailed to Marshak	1
California Institute of Technology (R. F. Bacher)	1
TOTAL	139

Information Division
Radiation Laboratory
University of California
Berkeley, California

UCRL-824
ABSTRACT

PAPER CHROMATOGRAPHY OF STEROIDS

David Kritchevsky and M. Calvin

Radiation Laboratory and Department of Chemistry,
University of California, Berkeley (*)

ABSTRACT

July 28, 1950

A method for the paper chromatography of sterols involving impregnated paper has been developed.

For publication in The Journal of the American Chemical Society

---

(*) The work described in this paper was sponsored by the Atomic Energy Commission.

PAPER CHROMATOGRAPHY OF STEROIDS[1]

David Kritchevsky and M. Calvin

Radiation Laboratory and Department of Chemistry,
University of California, Berkeley, California

The separation of cholesterol and cholestenone has been achieved by using paper impregnated with "Quilon"[2] as the stationary phase and simple primary alcohols as solvents. In effect, the stationary phase consists of the stearic acid residues.

Use of paper impregnated with rubber latex[3], silicic acid[4] and alumina[5] in paper chromatography has been reported. Of these, alumina paper was tried and found to give erratic results. The method of Zaffaroni and co-workers[6] for the paper chromatography of steroids using paper saturated with formamide

---

(1) The work described in this paper was sponsored by the Atomic Energy Commission.

(2) Stearato chromic chloride, generously supplied by E. I. duPont de Nemours and Company, Inc.

(3) Boldingh, Experientia, 4, 270 (1948).

(4) Kirchner and Keller, J. Am. Chem. Soc., 72, 1867 (1950).

(5) Datta and Overell, Biochem. J., 44, xliii (1949).

(6) Zaffaroni, Burton and Keutmann, Science, 111, 6 (1950).

or propylene glycol as the stationary phase and a hydrocarbon solvent was also tried. In these experiments the steroids were found to move with the front. Using ordinary paper (Whatman No. 1), cholesterol was found either to move with the solvent front or remain at the origin.

For ease of location, tritiated cholesterol was used and the material located by scanning the paper with a windowless counting tube designed to locate weakly radiating substances on paper[7].

The presence of cholesterol at the points of high activity was confirmed by the red color developed after papers treated with a solution of silicotungstic acid were dried[8]. Cholestenone gave an olive green color with this reagent, but only when the steroid was present in relatively large amounts. Cholestenone was most easily detected by the yellow color obtained with a reagent consisting of a solution of iodine and potassium iodide in water[9].

The most satisfactory solvents, to date, have been methanol, ethanol, and ethanol-water 8:2. The latter solvent gives the best separation of cholesterol and cholestenone. The results are tabulated below.

Table 1

Solvent	Cholesterol (R_f)	Cholestenone (R_f)
Methanol	0.56	0.77
Ethanol	0.92	0.97
80% Ethanol	0.52	0.86

(7) Gray, Ikeda, Benson and Kritchevsky, Rev. Sci. Inst., in press.

(8) Montignie, Bull. soc. chim., 51, 690 (1932).

(9) Munier and Macheboeuf, Bull. soc. chim. Biol., 31, 1144 (1949).

All experiments were carried out as descending chromatograms using 1-1/2" x 15" strips of the impregnated paper. The paper was usually wet to a distance of about 25 cm. from the origin. R_f values were measured from the farthest point of the origin and the foremost point of the colored or active zone.

Projected work includes widening the range of usable solvents, development of supplementary color reactions and extension of this method to other steroids.

SUMMARY

A method for the paper chromatography of sterols involving impregnated paper has been developed.