PAPER CHROMATOGRAPHY OF WEAKLY POLAR STEROIDS

ADDITIONS TO THE METHOD OF NEHER AND WETTSTEIN

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INTRODUCTION

In 1952 NEHER AND WETTSTEIN[1] published a method for separating weakly polar steroids by paper chromatography. A very good separation of many compounds can be achieved with this method, but constant results are only obtained with great difficulty. Other methods were published later by MICHALEC[2] and very recently by MARTIN[3]. Since MICHALEC's method did not give very satisfactory results in the separations the authors wanted to study, the conditions governing the separation by the method of NEHER AND WETTSTEIN were studied.

EXPERIMENTAL

Strips, 42 × 6 cm, were cut from sheets of Whatman No. 1 paper parallel to the machine direction, soaked in a 10% solution of phenylcellosolve in ether, removed from this solution at zero time and blotted between filter paper. Steroids were applied as solutions in ether and the strips exposed to the air at room or other temperature ("temperature of application") until a fixed time starting at zero time had elapsed ("time of application", usually 15 minutes). Immediately afterwards the strips were transferred to the chromatography tanks. Time of running was 135 minutes, ascending technique being used; the mobile phase was heptane saturated with phenylcellosolve. Steroids were detected in the usual manner[4].

RESULTS

The conditions described were varied and their influence on the R_F values of various compounds determined.

(1) The amount of phenylcellosolve in the filter paper largely determines the reproducibility of the R_F values. Optimal results were obtained when the paper contained about 15% phenylcellosolve at the end of the time of application. The influence on the R_F values is shown in Table I.

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TABLE I

INFLUENCE OF THE AMOUNT OF PHENYLCELLOSOLVE IN THE FILTER PAPER ON THE SEPARATION OF CHOLESTEROL AND CHOLESTENONE

Phenylcellosolve %		Distance front travelled in 135 min (cm)	Δ^4-Cholesten-3-one R_F	Cholesterol R_F
in ether (v/v)	in paper (w/w)			
2.0	1.9	22.3	0.83	0.90
4.0	6.0	22.8	0.70	0.88
8.0	13.4	22.7	0.71	0.82
10.0	14.8	21.8	0.71	0.79
16.0	19.5	18.8	0.63	0.68
20.0	22.3	16.0	0.55	0.41

TABLE II

INFLUENCE OF TIME AND TEMPERATURE OF APPLICATION ON THE R_F VALUES OF VARIOUS COMPOUNDS
A = cholesterol; B = 7α-hydroxycholesterol; C = Δ^4-cholestene-3,6-dione; D = cholestane-3,6-dion-5α-ol; E = cholesterol palmitate.

No.	Time (min)	Temp. °C	R_F				
			A	B	C	D	E
1	10	19	0.65	—	0.28	0.10	—
2	15	0	0.45	0.04	0.24	0.06	0.66
3	15	19	0.71	0.08	0.32	0.11	front
4	15	24	0.79	0.11	0.36	0.14	front
5	20	19	0.92	—	0.35	0.13	—
6	30	19	front	—	0.41	0.14	—

(2) The temperature and time of application had a marked influence on the separation, which is illustrated by Table II. The R_F values of all the compounds used showed a tendency to increase with rising temperature and increasing time of application.

The temperature during the actual chromatography did not have any appreciable influence on the separation.

(3) Mixtures of steroids of very low polarity (cholesterol, cholestanol and cholesterol esters) could be separated on Whatman Nos. 7 and 4, steroids of slightly higher polarity (cholesterol, Δ^4-cholesten-3-one) were preferably separated on Nos. 1 or 3. Mixtures of still higher polarity (steroid diols, diones) were separated by using the solvent system methylcellosolve-heptane instead of phenylcellosolve.

DISCUSSION

It is evident from the data given that the amount of phenylcellosolve in the paper, the time and the temperature of application should be kept strictly constant in order to obtain identical results. NEHER AND WETTSTEIN have mentioned the possibility that the impregnated paper could absorb water during exposure to the humid

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air—we, however, have never been able to demonstrate any influence of the relative humidity of the air on the separation. A few experiments with paper strips exposed to water vapour *before* impregnation showed that R_F values do increase when water is added to the system.

The influence of time and temperature of application on the separation provides a way of resolving mixtures of steroids of quite different polarity.

Table II (Nos. 2–4) shows that a mixture of very weakly polar steroids is separated when the time of application is short (15 min or less) and the whole procedure is performed in the cold room. A mixture of steroids of higher polarity is separated under the opposite conditions (Table II, Nos. 5 and 6), when the time of application is 15–30 min and the temperature is 19° or higher.

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SUMMARY

Some important conditions governing the separation of weakly polar steroids by paper chromatography according to the method of NEHER AND WETTSTEIN have been studied. It was shown that the reproducibility of the results was largely determined by the amount of cellosolve in the paper. Separation of mixtures of compounds of different polarity could be achieved by varying the conditions to which the impregnated paper was exposed before chromatography.

REFERENCES

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