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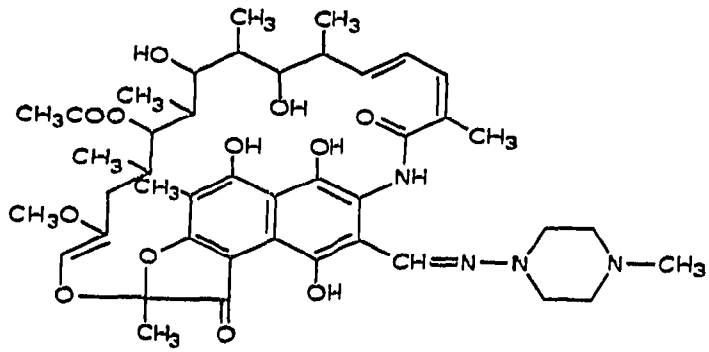
A simple thin-layer chromatographic method for the separation and identification of Rifampin and its metabolites

The drug 3-(4-methylpiperazinyliminomethyl)rifamycin SV has been introduced into human therapy under the name of Rifampin (USAN), and similar ones[1]. Since it is excreted both unchanged and as its desacetyl derivative[2], the need arose for a method for their separation and identification which is applicable in non-specialized laboratories.



Rifampin

Experimental and results

The separation and identification was done by thin-layer chromatography (TLC) on Eastman Chromagram Sheets No. 6060, coated with a 100-μm layer of silica gel, directly, without activation. The solvent system chloroform–ethanol–0.1 *N* HCl (84:15.9:0.1) was found to give the most suitable results.

Chloroform solutions of 1.0 mg/ml of both Rifampin and desacetylrifampin were prepared. The corresponding amounts (10-μl preferably) were placed on the starting points and developed once in one direction. The components were identified by their individual colours.

The following R_F values were found at room temperature (23°): Rifampin, 0.55; desacetylrifampin, 0.37.

The amount of material has a considerable influence on the R_F values. The quality of the spots formed by more than 20-μg disimproves. Thus, the use of corresponding standard samples is recommended (Table I).

In the case of urine specimens, chloroform extracts were prepared, concentrated

TABLE I

INFLUENCE OF AMOUNT OF RIFAMPIN AND DESACETYLRIFAMPIN ON THE CORRESPONDING R_F VALUES

Compound	*Amount*		
	2–20 µg	*60 µg*	*100 µg*
Rifampin	0.55	0.60	0.64
Desacetylrifampin	0.37	0.45	0.50

to approximately the same values and chromatographed as above. No interfering materials were found.

For estimation of eventual changes during the TLC, the spots were cut out after developing, eluted into 0.01 *N* NaOH[3] and scanned on a Hitachi–Perkin-Elmer Spectrophotometer Model 224. The curves between 220 and 650 nm showed identical patterns before and after chromatography, corresponding with those published previously[3].

One further very light spot, caused apparently by very slight oxidation, was found consistently on chromatograms of standard samples as well as on urine-extracted specimens[4]. As the whole procedure lasts no longer than three-quarters of an hour, the oxidation is kept to insignificant levels under these conditions.

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