COMPARISON THE SEPARATION OF FOUR SELECTED IMMUNOSUPPRESSANTS IN DIFFERENT THIN-LAYER CHROMATOGRAPHIC CONDITIONS

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INTRODUCTION

Among the various analytical methods, TLC thin-layer chromatography is still very popular and useful. It allows the analysis of multiple compounds simultaneously, it means on a single chromatographic plate [1]. In this study, four immunosuppressive compounds were analyzed: everolimus, zotarolimus, tacrolimus and temsirolimus. Thin-layer chromatography was used, in which mixtures of different organic solvents were applied as mobile phases and different chromatographic plates were utilized as stationary phases. The compounds studied are some of the most widely administered drugs in therapies to prevent transplant rejection and in various oncology therapies. Zotarolimus is a relatively new immunosuppressive drug used in stents. The structural formulas of the tested substances are shown in Figure 2.

METHODS AND MATERIALS

In this work, different TLC mobile phases were used, such as toluene/acetonitrile/acetic acid (6:4:0.1 v/v/v; F1), chloroform/toluene/methanol/acetic acid (4:4:2:0.1 v/v/v/v; F2), propan-2-ol/water (4:1 v/v; F3), acetonitrile/methanol/propan-2-ol (4:3:3 v/v/v; F4), ethanol/water (4:1 v/v; F5), acetonitrile/water (4:1 v/v; F6), methanol/water/formic acid (36:14:0.1 v/v/v; F7) and chromatography plates such as silica gel 60 F_{254} with and without concentrating zone, silica gel 60 with kieselguhr F_{254}, silica gel RP-18 F_{254}. Previous scientific reports describe mainly conditions for TLC analysis of each immunosuppressive compound tested separately and there is no data for analysis of four compounds tested simultaneously [2-4]. Based on the obtained retardation factors of the tested compounds, separation parameters such as ΔR_f, R_s, R_f^α and α were determined for all pairs of studied compounds. This parameters were successfully used to evaluate the suitability of the tested chromatographic systems for separation and identification of the examined compounds occurring simultaneously i.e. in one sample.

RESULTS

Analyses of all determined separation factors for studied compounds allowed to show the best chromatographic system for separation and next confirmation the identity of studied compounds. Of all the chromatographic systems tested, the most efficient one for the separation of four immunosuppressive compounds was the system consisted of the mobile phase F4 (acetonitrile/methanol/propan-2-ol 4:3:3 v/v/v), and the stationary phase was silica gel RP-18 F_{254} plates. Under this TLC conditions, the highest values of separation factors were achieved. In addition to this, all chromatographic bands are compact and easy to analysis. Excellent results ensuring complete separation and rapid identification was observed especially for a pair of tacrolimus and temsirolimus. Results of the study are shown in Table 1. A photo of the separation of the tested substances with the use of a chromatographic system consisting of a silica gel 60 with kieselguhr F_{254} and a mobile phase of toluene/acetonitrile/acetic acid (6:4:0.1 v/v/v; F1) is presented in Figure 1.

TLC Mobile phase	TLC Stationary phase	Everolimus and Zotarolimus separation				Zotarolimus and Tacrolimus separation				Tacrolimus and Temsirolimus separation			
		ΔR_f	R_s	R_f^α	α	ΔR_f	R_s	R_f^α	α	ΔR_f	R_s	R_f^α	α
F1	silica gel 60 F_{254}	0,03	3,14	1,05	1,14	0,13	3,20	1,24	1,73	0,22	3,64	1,69	2,49
	silica gel 60 F_{254} / kieselguhr	0,04	1,40	1,06	1,19	0,11	1,20	1,20	1,60	0,23	2,67	1,70	2,58
	silica gel 60 F_{254} with conc. zone	0,01	1,33	1,01	1,05	0,15	1,82	1,28	1,90	0,31	2,38	2,35	3,93
F2	silica gel 60 F_{254}	0,00	2,13	1,00	1,00	0,14	2,77	1,22	1,99	0,03	1,38	1,05	1,14
	silica gel 60 F_{254} / kieselguhr	0,00	1,42	1,00	1,00	0,14	2,35	1,22	1,97	0,03	2,15	1,05	1,14
	silica gel 60 F_{254} with conc. zone	0,01	0,97	1,01	1,07	0,16	2,27	1,25	2,25	0,04	1,18	1,07	1,19
F3	silica gel 60 F_{254} RP-18	0,03	1,36	1,06	1,13	0,11	1,79	1,24	1,56	0,53	1,65	2,15	1,16
F4	silica gel 60 F_{254}	0,04	1,43	1,04	2,43	0,01	2,22	1,01	1,18	0,01	2,00	1,01	1,21
	silica gel 60 F_{254} / kieselguhr	0,04	1,20	1,04	2,43	0,03	1,76	1,03	30,90	0,00	2,27	1,00	1,00
	silica gel 60 F_{254} RP-18	0,06	1,43	1,08	1,37	0,07	1,09	1,10	1,43	0,25	3,00	1,36	8,14
F5	silica gel 60 F_{254} RP-18	0,04	1,13	1,07	1,18	0,10	1,62	1,17	1,55	0,01	2,17	1,01	1,05
F6	silica gel 60 F_{254} RP-18	0,05	1,29	1,16	1,25	0,26	1,58	3,60	5,06	0,51	5,43	6,10	14,08
F7	silica gel 60 F_{254} RP-18	0,01	2,50	1,33	1,35	0,03	2,00	4,00	4,13	0,03	1,76	4,00	4,13

Table. 1 Separation parameters of tested compounds.

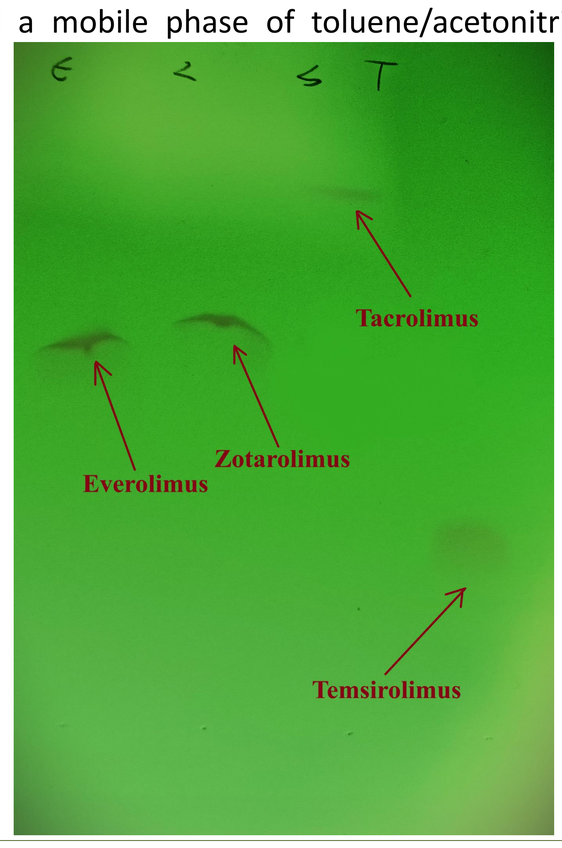
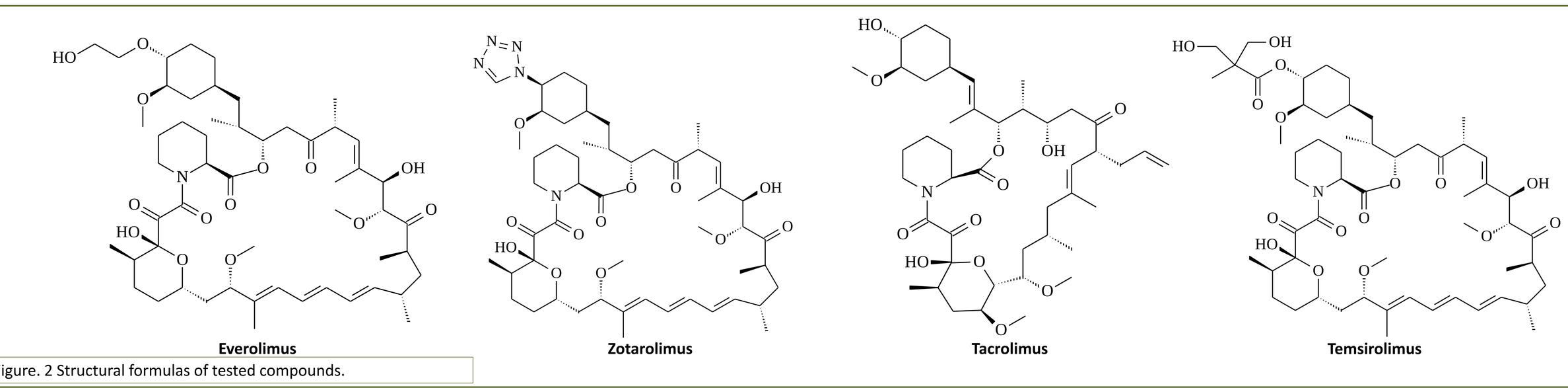


Figure. 1 Picture of TLC chromatogram developed on silica gel 60 with kieselguhr F_{254} chromatographic plate and a mobile phase consisted of toluene/acetonitrile/acetic acid (6:4:0.1 v/v/v; F1)



Everolimus **Zotarolimus** **Tacrolimus** **Temsirolimus**

Figure. 2 Structural formulas of tested compounds.

CONCLUSIONS

The proposed TLC conditions showed promising results and may be applicable to quality control of drug substances under study (rapid separation and identity test). The developed method is fast and economical, because it allows the analysis of four tested compounds simultaneously. It was stated that of all chromatographic systems studied, the best for separation and identification is the one composed of the mobile phase F4 (acetonitrile/methanol/propan-2-ol, 4:3:3 v/v/v) and silica gel RP18 F_{254} plates.

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Acknowledgements

The research was carried out as part of a project financed by the Medical University of Silesia in Katowice No. BNW-2-022/N/3/F and BNW-2-057/N/4/F.