The Determination of Endo- and Exogenic Steroids in Biological Liquids by Thin-Layer Chromatography Methods

Kartsova L.A.*, Strelnikova E.G.**

**** Saint-Petersburg State University***
***** Medical Academy of Postgraduation Study, Saint-Petersburg***
lstrelnikova@inbox.ru

1. Introduction

The HPTLC are enough convenient for laboratory express diagnosis. We perfomed the separation of exo- (drugs: prednisolone, cortisone acetate, dexamethasone and dexamethasone phosphate) and endogenic corticosteroids (cortisol, cortisone, corticosterone, 11-deoxycorticosterone and 11-deoxycortisol) using HPTLC (classical and micellar modes) with UV and videodensimetric detection.

2. Experimental

The cardinal problem for the qualitative and quantitative identification is the nearness of chemical structures (Fig. 1).

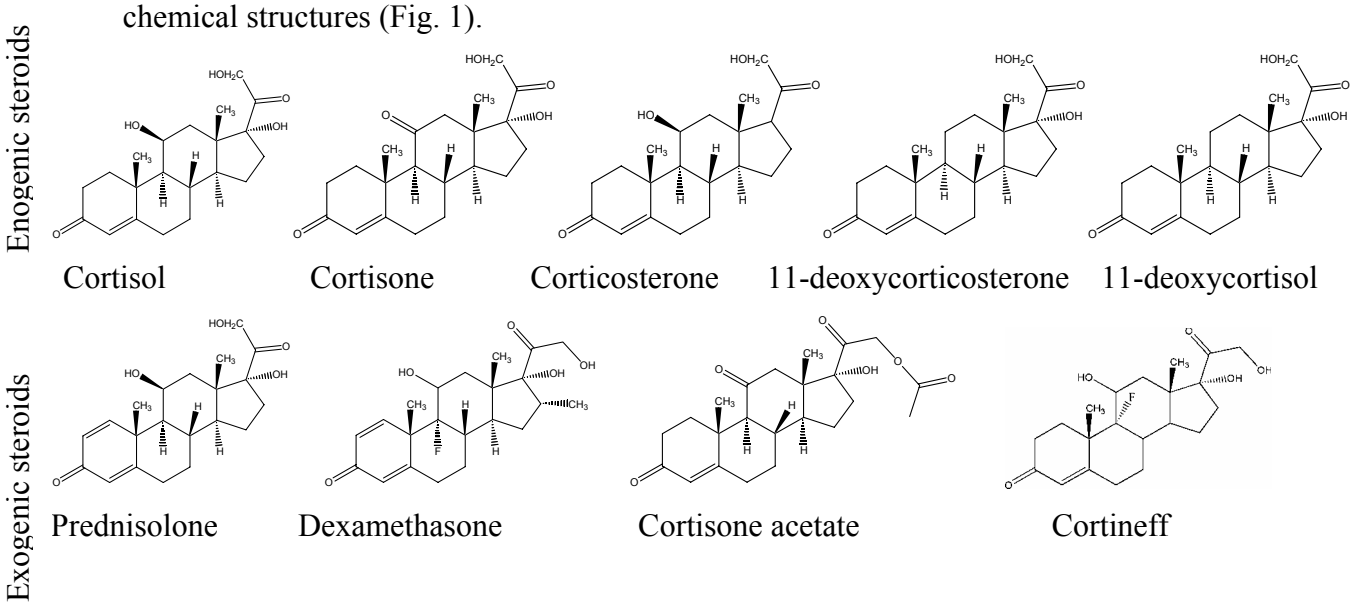

Fig.1. Steroids structures

The conditions of their separation were optimized on the model sample steroids solution. Non-aqueous mobile phases with different contents such as hexan-ethyl acetate (3:7, v/v), benzene-acetone (4:1, v/v), benzene-ethanol (4:1, v/v), benzene-ethyl acetate (3:7, v/v), toluene-ethanol (9:1, v/v), chloroform-methanol (47:3, v/v), chloroform-ethanol (9:1, v/v) were tested. Thus the selected by us phases (hexan-ethyl acetate (3:7, v/v), benzene-ethanol (9:1, v/v), toluene-ethanol (9:1, v/v)) were found the most successful. Using double elution by this eluents

(or their combination) we succeeded in absolute separation of analyzed substances (Fig. 2). TLC was performed on high performance plates with 8 – 12 μm granulations and 100 μm layer thickness (*«Lenchrom», Saint-Petersburg*). The detection limits were determined for each component and comes to 100 ng/ml.

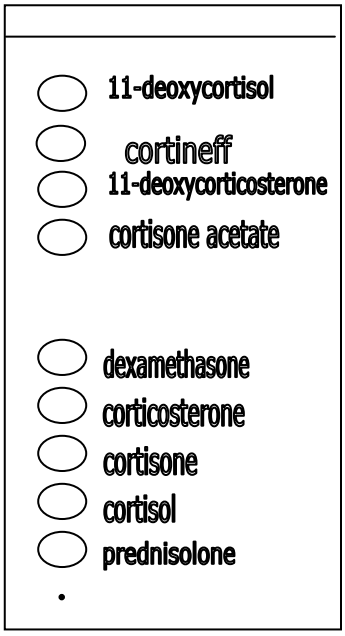

Fig. 2. The scheme of separation of endo- and exogenic steroids by HPTLC (eluent: the first elution – toluene-ethanol (9:1, v/v), the second elution – hexan-ethyl acetate (3:7, v/v)).

Besides, the minimum of organic content in the mobile phase is preferably for the use of such scheme of analysis in the practise of klinical medicine. The application of water was unsuccessful. We suggested the water-micellar solution of SDS could explain this role. It was illustrated on the example one of drugs – prednisolone, as most hydrophilic and most strongly adhered to the plate. The optimal mobile phase was the water solution with the addition of 15 mM sodium dodecylsulfate (Fig. 3).

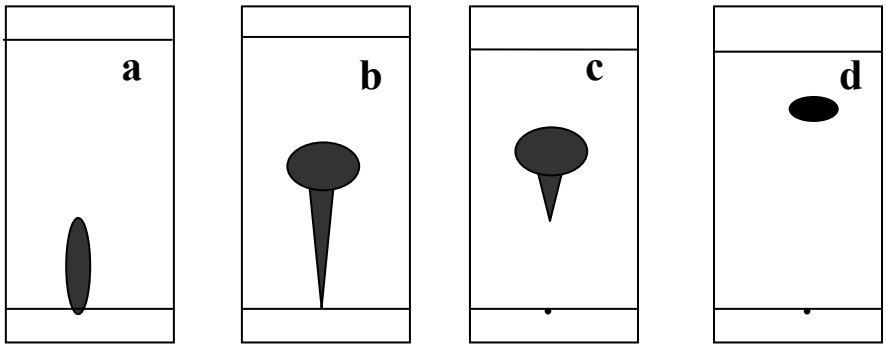

Fig. 3. The scheme of determination of prednisolone by HPTLC. Eluents: water a) without addition and with b) 5 mM SDS, c) 8,3 mM SDS, d) 15 mM SDS

Nevertheless the addition of negligible quantities of organic solvents (acetonitrile) in the water-micellar mobile phase allows increase of efficiency and separation of analytes (Fig. 4).

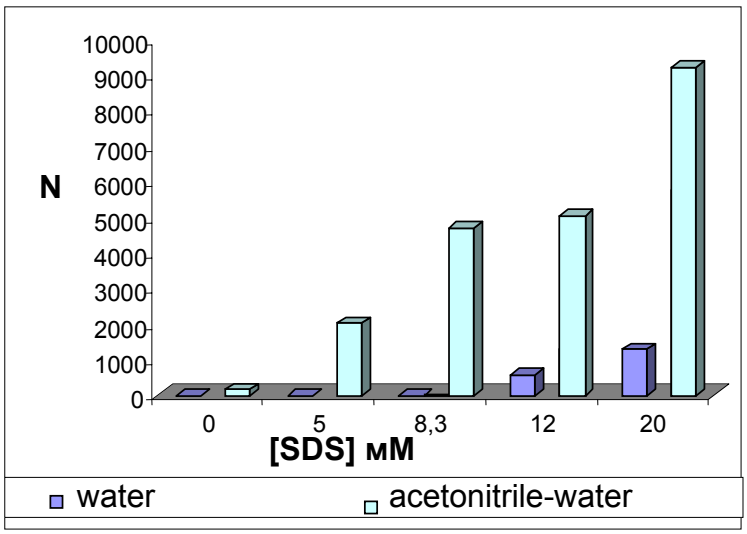

Fig. 4. The influence of SDS addition on the efficiency (the number of theoretical plates), calculated for the cortisone

3. Conclusion

So proposed phases (either organic or water-micallar) allows determining of endo- and exogenic corticosteroids concurrently. The developed systems are suitable not only for the revealing of residual drugs in biological liquids but for the estimation of the purity of drugs.

Acknowledgements

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