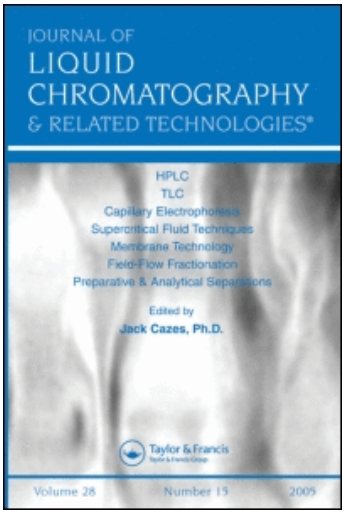

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Corticosteroid Analysis by HPLC with Increased Sensitivity by Use of Precolumn Concentration

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CORTICOSTEROID ANALYSIS BY HPLC WITH INCREASED SENSITIVITY BY USE OF PRECOLUMN CONCENTRATION

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ABSTRACT

In this paper ttwo HPLC methods are described which offer the possibility of increasing the sensitivity for a couple of corticosteroids The increase is achieved by precolumn concentration with C-18 cartridges from 5 to 20 fould. The concentration step is in principle applicable to all other steroids.

INTRODUCTION

Many HPLC methods for determination of corticosteroids have been reported (1-7). For drug release testing of topical forms extremely sensitive methods are required. In this paper the possibility of increasing the sensitivity by precolumn concentration is described.

MATERIALS

Hydrocortisone, Hydrocortisone-21-acetat, Prednisolon, Dexamethason, Betamethasone-17-valerate and Flumethason-21-acetat were from Sigma (St.Louis, USA). C-18 Cartridges, Bond elut, Vac-elut by Analytichem International I.C.T.; all solvents are of HPLC grade.

EQUIPMENT

HPLC: Perkin Elmer Series 10; detector: Perkin Elmer LC 235, automatic sample injector: ISS-100 Perkin Elmer, Software: Omega-2 Vers.2.50, Column: Nucleosil C-18 (150x4 mm ID), 5 µm particle size.

METHODS

Assay procedure:
A Bond elut C-18 cartridge, which has been prepared for use by rinsing with 4 ml of water followed by 3 ml of methanol, is inserted onto a vac-elut system. An aliquot of the aqueous corticosteroid solution is added and sucked through the cartridge. The corticosteroid is absorbed by the apolar sorbent and is eluted from the cartridge with less methanol than the aqueous corticosteroid solution. The concentration was increased from five to twenty fould.
The precolumn concentration offers the possibility of increasing the sensitivity more than 20 fould.
Method 1:
The mobile phase consisted of a CH_3OH/H_2O (7+3). The flow rate was set 1,0 ml/min. After precolumn concentration as described before the injection volume for the sample preparations was maintained at 20 µl. This method was used for Hydrocortison (240 nm), Hydrocortison-21-acetat (240 nm) and Prednisolon (242 nm). The corticosteroids were quantitated by comparing the peak area of the

TABLE 1
Recovery of Hydrocortison (HC), Hydrocortisonacetat (HCac) and Prednisolon (Pred) after precolumn concentration (method 1)

HC µg/ml spiked	5 fould conc. µg/ml	Recovery %	µg/ml
1.46	7.3	110	8.0
1.44	7.2	102	7.3
1.34	6.7	93.5	6.32

HCac µg/ml spiked	10 fould conc. µg/ml	Recovery %	µg/ml
0.55	5.5	105,4	5.8
0.65	6.5	103.1	6.7
0.85	8.5	94.1	8.2

Pred µg/ml spiked	10 fould conc. µg/ml	Recovery %	µg/ml
0.835	8.35	104.1	8.7
0.64	6.4	101.2	6.4
0.504	5.04	98.5	5.1

unknown with standard curves (for each corticosteroid 5 points) prepared by adding known amounts of each corticosteroid. Standard solutions containing 4-14 mg/ml. Linear regression analysis of the peak areas gave correlation coefficients (r) between 0,98 and 0,99. The detection limit was 3,5µg/ml.

Method 2:
The mobile phase consisted of acetonitril/H_2O (7+3). The flow rate and injection volume were the same as in method 1.

TABLE 2
Recovery of Dexamethason (Dex), Flumethason-21-acetat (Flu) and Betamethason-17-valerat (Bet) after precolumn concentration (method 2)

Dex µg/ml spiked	10 fould conc. µg/ml	Recovery %	µg/ml
0.6	6.0	105.5	6.33
0.33	3.3	117.3	3.87
0.3	3.0	88.4	2.65

Flu µg/ml spiked	20 fould conc. µg/ml	Recovery %	µg/ml
0.14	2.8	91.4	2.56
0.115	2.3	100.0	2.3
0.067	1.35	108.1	1.46

Bet µg/ml spiked	20 fould conc µg/ml	Recovery %	µg/ml
0.285	5.7	105	5.98
0.180	3.6	98.1	3.52
0.045	0.9	88.9	0.8

This method was used for Dexamethason (239 nm), Betamethason-17-valerate (239 nm) and Flumethason-21-pivalat (237 nm). The corticosteroids were quantitated by comparing the peak area of the unknown with standard curves (for each corticosteroid 5 points) prepared by adding known amounts of each corticosteroid. Standard solutions containing 3-18 mg/ml. Linear regression analysis of the peak areas gave correlation coefficients (r) between 0,98 and 0,99. The detection limit was 0,12 µg/ml.

RESULTS

Recovery:

Recovery data for the solid phase extraction sample preparation were generated by spiking acceptorphase (phosphate buffer 10 mM, pH 7,4, 150 mM sodium chloride) with known amounts of the corticosteroids. The recovery rates are listed in table.1 and 2.

DISCUSSION

For release experiments of corticosteroids from topical forms (ointments, gels, lotions) extremely sensitive analytical methods are required. The corticosteroid in the aqueous acceptor phase may be less than 0,5 µg/ml for Hydrocortison, Hydrocortison-21-acetat and Prednisolon and about 0,02 µg/ml for Betamethasone-17-valerate and Flumethason- 21-acetat. Solid phase C-18 cartridges were used to concentrate the corticosteroids till 20 fould. The concentration step is in principle applicable to all other steroids.

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