This article was downloaded by: On: *25 January 2011* Access details: *Access Details: Free Access* Publisher *Taylor & Francis* Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK

### Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information: http://www.informaworld.com/smpp/title~content=t713597273

# HPLO TLC Capitality Electrophonesis Supercritical Fluid Techniques Mentorane Technology Field-Fow Fractionation Preparative 8, Analytical Separations Eletes by Jack Cazes, Ph.D.

CHROMATOGRAPHY

& RELATED TECHNOLOGIES

LIQUID

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

C. Valentaª; H. Janoutª ª Institute of Pharmaceutical Technology, University of Vienna, Vienna, Austria

To cite this Article Valenta, C. and Janout, H.(1994) 'Corticosteroid Analysis by HPLC with Increased Sensitivity by Use of Precolumn Concentration', Journal of Liquid Chromatography & Related Technologies, 17: 5, 1141 – 1146 To link to this Article: DOI: 10.1080/10826079408013391 URL: http://dx.doi.org/10.1080/10826079408013391

## PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

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

C. VALENTA AND H. JANOUT

Institute of Pharmaceutical Technology University of Vienna Währinger Straße 25 1090 Vienna, Austria

#### ABSTRACT

In this paper two 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-21acetat 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

#### CORTICOSTEROID ANALYSIS BY HPLC

### TABLE 1

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

НС	5 fould conc.	Recovery	
µg/ml spiked	µg/ml	%	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	10 fould conc.	Recovery	
µg/ml spiked	µg/ml	%	µg/m]
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	10 fould conc.	Recovery	
µg/ml spiked	μg/ml	%	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  $3.5\mu g/ml$ .

### 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	20 fould conc.	Recovery	
µg/ml spiked	µg/ml	%	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	20 fould conc	Recovery	
µg/ml spiked	µg/ml	96	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  $\mu$ g/ml.

#### CORTICOSTEROID ANALYSIS BY HPLC

#### 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  $\mu$ g/ml for Hydrocortison, Hydrocortison-21-acetat and Prednisolon and about 0,02  $\mu$ g/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.

#### REFERENCES

1. J.A.Mollica, R.F.Strusz, J.Pharm.Sci., 61: 444-447 (1972)

2. M.C.Olson, J.Pharm.Sci., 62: 2001-2006 (1973)

3.A.R.Lea, J.M.Kennedy, G.K.-C. Low, J.Chromatogr., <u>198</u>: 41-47 (1980)

4. F.Huber, M.Wiedemann, G.Heinrich, Z.Salama, H.Jaeger, Drug Res., <u>40</u>: 926-931 (1990)

5. J.Girault, B.Istin, J.M. Malgouyot, A.M.Brisson, J.B.Fourtillan, J.Chromatogr. <u>564</u>: 43-53 (1991)

6.G.R.Cannell, R.H. Mortimer, D.J. Maguire, R.S.Addison, <u>563</u>: 341-347 (1991)

7. J.Noma, N.Hayashi, K.Sekiba, J.Chromatogr. 568: 35-44 (1991)

Received: August 21, 1993 Accepted: August 31, 1993