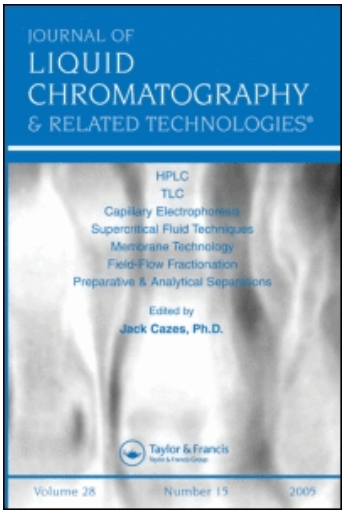

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Improved High Performance Liquid Chromatographic Method for the Determination of Some Corticosteroids

A. Shalaby[a]; M. Shahjahan[ab]
[a] Pharmaceutical Chemistry Department, Faculty of Pharmacy Zagazig University, Zagazig, Egypt [b] Department of Pharmaceutics, the School of Pharmacy University of London, London, England

To cite this Article Shalaby, A. and Shahjahan, M.(1991) 'Improved High Performance Liquid Chromatographic Method for the Determination of Some Corticosteroids', Journal of Liquid Chromatography & Related Technologies, 14: 7, 1267 — 1274
To link to this Article: DOI: 10.1080/01483919108049319
URL: http://dx.doi.org/10.1080/01483919108049319

JOURNAL OF LIQUID CHROMATOGRAPHY, 14(7), 1267-1274 (1991)

IMPROVED HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF SOME CORTICOSTEROIDS

A. SHALABY[1] AND M. SHAHJAHAN[2]

[1]Pharmaceutical Chemistry Department
Faculty of Pharmacy
Zagazig University
Zagazig, Egypt
[2]Department of Pharmaceutics
The School of Pharmacy
University of London
London, England

ABSTRACT

A simple, selective and accurate high performance liquid chromatographic method for the determination of some pharmaceutically important corticosteroids has been developed. The suggested method uses a ultrasphere ODS column with acetonitrile-phosphate buffer (pH 8) as a mobile phase.
The mean percentage recovery ranged from 97.9 to 99.7. The proposed method was applied to the determination of the studied corticosteroids in some dosage forms. The statistical analysis of the results obtained were compared favourably with those given with the official method.

* Correspondance

INTRODUCTION

The efficiency of corticosteroids for the treatment of inflammatory conditions is well established and many formulations containing corticosteroid derivatives are available in the market. Recent interest has been shown in the development of high performance liquid chromatographic (HPLC) method for many pharmaceuticals, because even with relatively short columns, very difficult separations can be accomplished with this method due to its greater selectivity. Greater selectivity is achieved through choice of mobile phase, solvent programming and variation of separation mechanism. Various HPLC methods have been reported (1-10) for the determination of some corticosteroid derivatives in pharmaceutical preparations and biological fluids. All these methods were applied only to a single corticosteroid derivative both in biological fluid or in dosage form.

In this paper, attempt has been made to develop a simple HPLC method based on reversed phase, isocratic elution and a variable wavelength UV detector which can be applied for the determination of corticosteroids in the presence of other compounds after simple extraction form its dosage form. The following representative examples were employed: hydrocortisone, hydrocortisone acetate, dexamethasone, prednioslone and prednisolone acetate in pure as well as in dosage forms.

EXPERIMENTAL

I. Materials:

Prednisolone, hydrocortisone, dexamethasone, prednisolone acetate and hydrocortisone acetate were kindly provided by various manufacturers and were used as received. Pharmaceutical preparations containing the compounds were randomly obtained from commercial sources. All reagent chemicals were of analytical grade and used without further purification. Solvents used were of HPLC grade.

II. HPLC method:

(a) Apparatus and operating conditions:

A Beckman model 334 gradient HPLC system, model 165 UV detector and a Hewlett-Packard integrator (model 3392 A) were used. The

column used was ultrasphere ODS (4.6 mm X 150 mm) 5 um. The mobile phase composed of 6:4 (v/v) mixture of acetonitrile and phosphate bufer (pH 8) and was degassed before use. Detection wavelength varied between 240-250 nm. Flow rate was 1.5 ml/min. Routinely, the system was allowed to equilibrate (approximately 3 hours) until a steady base line was observed. 20 µl volume was injected with a fixed voulme loop valve system. The sample was injected until at least two reproducible peaks were obtained. To was tested by using H_2O_2.

(b) Sample preparation (pure form):

A stock solution containing the appropriate amound in methanol (Table 1) was prepared and diluted with methanol to give five different concentrations for each corticosteroid derivative.

(c) Sample preparation (dosage form):

1. Dexamethasone (Eye ointment 0.5 mg/gm)

10 gm ontment were taken to which 20 ml methanol added. put in a waterbath at 80°C for dispersion. The suspension was then placed in ultrasonic bath for extraction for 15 minutes. cooled. filtered and completed to 20 ml with methanol. This soultion was further diluted appropriately to give approximately dexamethasone concentrations of 0.1 mg/ ml. 0.2 mg/ml. 0.3 mg/ml . 0.4 mg/ml and 0.5 mg/ml.

2. Hydrocortisione (Lotion 10 mg/gm)

1 gm was extracted with 20 ml methanol in a similar manner as above. Finally the solution was diluted to obtain the following concentrations : 0.1 mg/ml 0.2 mg/ml , 0.3 mg/ml, 0.4 mg/ml and 0.5 mg/ml.

3. Hydrocortisione acetate (Ointment 2.5 mg/gm)

2 gm were extracted with 20 ml methanol in a manner similar to that of dexamethasone ointment. Dilution was made to obtain a concentration of 0.1 mg/ml, 0.2 mg/ml, 0.3 mg/ml, 0.4 mg/ml and 0.5 mg/ml.

4. Prednisolone (Tablet 10 mg/tablet)

Tablet powder equivalent to 10 mg active ingredient was extracted with 20 ml methanol using ultrasonic bath. After filtration, diluted to

get a concentration of 0.1 mg/ml, 0.2 mg/ml, 0.3 mg/ml , 0.4 mg/ml and 0.5 mg/ml.

5. Prednisolone acetate (Injection 20 mg/ml)
1 ml was diluted to 20 ml with methanol , put in a ultrasonic bath, and finally diluted to get different concentration of 0.1 mg/ml, 0.2 mg/ml , 0.3 mg/ml , 0.4 mg/ml and 0.5 mg/ml.

III. Official method:

The procedure for the official method was that of British **Pharmacopoea, 1988**) (Appendix VIII N)[11]. Beckman DU 70 UV / vis spectrophotometer has been used for absorbance measurements.

RESULTS AND DISCUSSION

A comprehensive study of a quality control system is excercised. In this work, we tried to estabish a high Performance liquid chromatographic system which can be generally applied for the identification and the quantitative determination of corticosteroid derivatives in pure form or in pharmaceutical preparations.

In this study the selectivity of different organic solvents : Methyl alcohol, acetoritrile and tetrahydrofuran have been tested separately after mixing with phosphate buffer at different pH and with different ratioes. Slight changes in pH (pH 7-8)appear to have little effect since retention times remained approximetly constent. The selectivity was howoever best at pH 8. All the tested analystes gave highly resolved, sharp peaks with acetonitrile-phosphate buffer at pH (8) 6:4 as mobile phase . The effect of temperature has not been thoroughly investigated, but generally the efficiency was maximum at $30^{o}C$ for all the tested compounds. In general, the capacity factor ($\bar{K}$) decreased with increasing the temperature. The effect of hydrophobicity of the stationary phase was not studied, however to overcome the problem of obtaining deviating results due to the use of different commercial stationary phases, a single column was used troughout all the experiments[12]

Table (1): Collective data for the analysis of corticosteroids in pure form.

Compound	Concentration of stock soln. mg/ml	Concentration mg/ml	Wavelength of detection
Dexamethasone	10.0	0.10-0.50	239 nm
Hydrocortisone	11.0	0.11-0.55	242 nm
Hydrocortisone acetate	8.5	0.085-0.425	242 nm
Prednisolone	12.0	0.12-0.60	242 nm
Prednisolone acetate	10.0	0.10-0.50	243 nm

Table (2): Some chromatographic parameters for corticosteroid derivatives.

Compound	$\bar{K}$	N	H
Prednisolone	0.647	4338	0.035mm
Hydrocortisone	0.695	4598	0.033mm
Dexamethasone	0.840	5416	0.028mm
Prednisolone acetate	1.067	6833	0.022mm
Hydrocortisone acetate	1.473	9787	0.015mm

$\bar{K}$: Capacity factor.
N : Number of theoretical plate.
H : Height equivalence of theoretical plate.

Talbe (3): Resolution and the relative retention of some mixtures of corticosteroid derivatives.

Corticosteroid derivative mixture	R_s	α
Prednisolone + Prednisolone acetate	2.52	1.65
Hydrocortisone + Hydrocortisone acetate	4.68	2.12
Hydrocortisone acetate + Dexamethasone	3.80	1.754
Prednisolone + Hydrocortisone acetate	4.96	2.28
Hydrocortisone + Prednisolone acetate	2.24	1.54

α : Relative retention.

R_s : Resolution factor

Table (4): Comparative analytical results of the proposed and official method for some corticosteroid derivatives in pure form.

Compound	% Recovery*	
	Proposed method	Official method
Dexamethasone	98.376 ± 0.717	98.214 ± 0.496
Hydrocortisone	99.620 ± 0.367	99.240 ± 0.837
Hydrocortisone acetate	98.401 ± 0.600	98.941 ± 0.488
Prednisolone	99.360 ± 0.613	99.180 ± 0.326
Prednisolone actate	99.194 ± 0.805	99.460 ± 1.013

* Average of five separate determinations.

Table (5) Comparative analytical results of the proposed and official method for some corticosteroid derivatives in some pharmaceutical preparations.

Compound	% Recovery*	
	Proposed method	Official method
Dexamethasone (ointment)	97.945 ± 0.817	98.040 ± 0.862
Hydrocortisone (lotion)	99.074 ± 0.773	99.094 ± 0.768
Hydrocortisone acetate (ointment)	99.400 ± 0.773	98.646 ± 0.768
Prednisolone (tablet)	99.544 ± 0.310	99.712 ± 0.356
Prednisolone acetate (injection)	99.623 ± 0.277	99.660 ± 0.356

* Average of five separate determinations.

Table (2) shows the efficiency of the applied system where the values of the theoretical plate (N) and the height equivalence of thearetical plate (H) indicate the good selectivity of this method. The efficiency for separating some mixtures of closely related corticosteroids has been tested. The resolution (Rs) and the relative retention (α) illustrated in Table (3) indicate the high resolution efficiency.

Table (4) shows the statistical data of the analytical results obtained by the proposed method and the official method for some corticosteroid derivatives in pure form. As evident, there is no significant difference between the two methods as regard to the accuracy and precison. The results obtained were encouraging and prompted us to apply the proposed method for the determination of these corteosteroid derivs in pharmaceutical products. The results in Table (5) show an agreement with those given with the official methods (within ± 0.8%) . The commonly used excipients, colours preservatives were found to offer no positive interference by the proposed method. Thus making the method more reliable,

less time consuming and more suitable for routine analysis in dosage forms and in biological fluids.

Despite the fact that the proposed method was not tried for the analysis of all corticosteroid derivatives, yet on the basis of the above criteria, we find that this method may be applicable to other corticosteroid derivatives.

REFERENCES

1. Rocci, M.L.Jr. and Jusko, W.J.J. Chromatogr. 1981. 224, 221
2. Rose, J.Q. and Jusko, W.J. J. Chromatogr. 1979, 162, 273
3. Devries, C.P., Lomaky-Janausek, M. and Poppsnijder, C. J. Chromatogr 1980, 183, 87
4. Goehl, J.J., Sundaresan, G.M., Hunt, J,P., Prasad, V.K., Toothaker, R.D. and Welling, P.G. J. Pharm. Sci., 1980. 69 1409
5. Tsuei, S.E. and Ashley, J.J J. Chromatogr. 1978. 145, 213.
6. Gupta, V.D. J. Pharm. Sci. 1979. 68, 908.
7. Scott, N.R. and Dioxon, P.F. J. Chromatogr. 1979. 164, 29.
8. Gupta, V.D. J. Pharm. Sci. 1979. 68, 926.
9. Lea, A.R. Kennedy, J.M. and Low, G.K.D. J. Chromatogr. 1980. 198, 41.
10. Rehm, K.D. Pharmaz . Ztg. (1981) 129, 99.
11. British Parmacepoea 1988 Vol. II Appendix VIII N PP A 124.
12. Smith , R.M. J. Chromatogr. (1982) 236, 321.