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Simultaneous determination of dexamethasone and trimethoprim by liquid chromatography

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Abstract

A reverse phase high performance liquid chromatography (HPLC) method to determine dexamethasone phosphate and trimethoprim is described in this paper. The separation was made in a LichrCART® C18 column using an acetonitrile–NaH_2PO_4 (10 mM) (70:30, v/v) (pH 3) buffer solution as mobile phase. The mobile-phase flow rate and the sample volume injected were 1 ml/min and 20 μl, respectively. The limits of quantification were about 0.25 mg/l for each compound. The method was applied in synthetic mixtures and in pharmaceutical formulations. Analyses were made by preparing test (from the stock solution) method whose results were compared with those obtained by means of the standard addition method. Both methods showed similar results, and then it was proved that some pharmaceuticals claimed levels were in agreement with the obtained results by using our analytical method.

Keywords: HPLC; Reverse phase; Dexamethasone phosphate; Trimethoprim

1. Introduction

Corticosteroids have been widely used as anti-inflammatories in medicine. Nowadays, pharmaceutical formulations contain corticosteroids in combinations with antibacterials since corticosteroids do not cure the fundamental cause of the disease by themselves (for example an infection) [1–3].

These compounds are very effective against a wide range of ocular, allergic and cutaneous inflammatory disease so there are a lot of formulations (dexamethasone–trimethoprim (DEX–TMP)) and concentrations in variable power for local administration.

The spectrophotometric methods for determination of DEX were based on colorimetric reactions [4]. High performance liquid chromatography (HPLC) methods are the most important. Both

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reverse-phase [5–7] and normal-phase chromatography [8,9] have been applied to the determination of DEX in plasma, coupled with UV detection generally at 254 nm. The fluorimetric detection is possible too, based on the conversion of the corticosteroids into the corresponding fluorescent derivatives with different chemical reactions [10,11].

There are examples of rapid and simple determination of DEX and associated compounds, in pharmaceutical formulations and in tears by means of capillary electrophoresis using a diode array detector. A selective capillary zone electrophoresis (CZE) micro assay was developed for the simultaneous determination of DEX phosphate and its major metabolite in tears [12,13]. Determination of DEX in association with some antibiotics, paracetamol and dapsone is possible by means of micellar electrokinetic chromatography (MEKC) [14]. Several work determinate mixtures of corticosteroids by MEKC in serum [15,16]. Other methodologies are described for their estimation including gas chromatographic [17] methods.

The spectrophotometric methods to determinate TMP were based on direct methods [18], colorimetric reactions [19], and derivative spectrophotometry [20,21]. There are some electrochemical methods for their direct determination [22]. Various techniques have been described for the analysis of TMP and sulfamethoxazole in biological systems, either individual or combined. The most widely used technique for simultaneous determination is HPLC [23–25].

There are examples of rapid and simple determination of TMP in plasma samples and associated with other compounds in pharmaceutical preparations by means of MEKC [26,27] and CZE [28–30] using a diode array as detector and using an amperometric detector for determination of different sulphonamides associated with TMP [31].

In this work, the separation of DEX and TMP through HPLC was studied. Since liquid chromatography offers important advantages, such as rapid set-up of instrumentation, versatility and low cost, and has proved to be a valuable method in the quality control of drugs, its performance in the separation of DEX and TMP has been evaluated in this paper. Satisfactory sensitivity, accuracy and precision were noted. The method provides a very short analysis time (5 min) and the LODs have been reduced.

2. Experimental

2.1. Apparatus

A Shimadzu L.C. model LC-10[a] with diode-array detector S.P.D. M10A, provided with a double pump system, which allows working in gradient mode, was used. It was also supplied with a Rheodyne Model 7725 injector with a 20 μl sample loop, and a Silicon 486/33 computer fitted with CLASS-LC 10 software, which was used for all the measurements and treatment of the data. The analytical column was a LichroCART (125 × 4.6 mm ID) containing LiChrospher 100 Rp-18e (particle size 5 μm). Before running a separation process, the mobile phase was always filtered by using Millipore filter kit. Dissolved gases were removed with a 10 min helium purge.

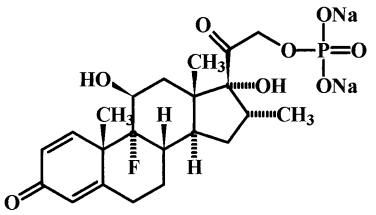
A Beckman Instrument DU-70 spectrophotometer, equipped with a 1.0 cm cell and connected to an IBM-PS model 30 computer, was provided with Beckman Data Leader Software (Fullerton, CA, USA).

A Crison (Barcelona, Spain) MicropH 2002 pH meter was used for pH measurements.

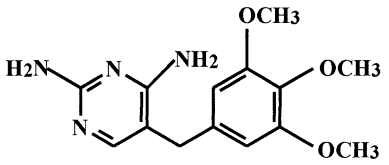
2.2. Reagents

All the solvents and reagents were of analytical grade unless indicated otherwise. Solutions were made with deionized water (Milli Q quality). Sodium salt of DEX phosphate and TMP, were from Sigma Chemical Co. (Germany and Switzerland). The stock solutions (100 mg/l) of DEX and TMP were prepared in deionized water and in a mixture ethanol:water (50:50; v/v). NaH_2PO_4, HCl and acetonitrile (LC grade) were from Panreac (Barcelona, Spain).

The stabilities of both analytes were evaluated over a period of 30 days by means of spectrophotometric measurements. The spectra of all compounds were recorded at a concentration level



DEXAMETHASONE PHOSPAHATE



TRIMETHOPRIM

Fig. 1. Chemical structure at the two compounds.

of 36 mg/l between 190 and 400 nm at a scan speed of 600 nm/min. Two compounds were assumed to be stable under operating conditions. The structure of these compounds is given in Fig. 1.

OTIX: this is an ocular-drop with DEX Phosphate disodium, Polymyxin B sulphate and TMP from the enterprise FarmaCusi S.A. OFTALMOTRIM-DEXA: This is an eye-drop with DEX Phosphate disodium, Polymyxin B sulphate and TMP from the enterprise FarmaCusi S.A. LIQUIPON-DEXAANTIBIÓTICO: this is an eye-drop with DEX phosphate disodium, Polymyxin B sulphate from the enterprise Iquinosa SA. OFTALMOTRIM: This is an eye-drop with TMP, Polymyxin B sulphate from enterprise FarmaCusi S.A.

2.3. Chromatographic conditions

Mobile phase	10 mM phosphate buffer (pH 3.0):acetonitrile (25:75)
Flow rate	1 ml/min
Temperature	25 °C
Detection wavelength	242 nm for DEX and 230 nm for TMP

3. Results and discussion

3.1. Optimization of separation conditions

3.1.1. Preliminary investigations

In this work, we have used the reverse phase liquid chromatography to study the separation of DEX and TMP on a LichroCART® C_{18} column.

A solution containing 8 mg/l of each one which shall be called "Z", was prepared by diluting the stock solution of the two compounds. Methanol and acetonitrile as organic solvent in the mobile phase were studied in order to find better selectivity and resolution, using phosphate as a buffer (pH 3.0; 10 mM) in the mobile phase.

The injection resulted in peak broadening when methanol was used, whereas in acetonitrile it did not, so acetonitrile was chosen as organic solvent in the composition of the mobile phase.

At first, a phosphate buffer (pH 3.0; 10 mM) was taken because an acid medium is usually employed in the separation of corticosteroids [6] and sulfonamides [24] and because the column works properly from pH 2.7 up to 7. At pH 3, DEX and TMP are charge.

Thus, the LichroCART® RP-18e C_{18} column, an acetonitrile–NaH_2PO_4 (10 mM) (70:30, v/v) (pH 3) as mobile phase and default selected flow-rate of 1 ml/min resulted to be the initial conditions for this chromatographic study.

3.1.2. Effect of the pH

The influence of the pH of the mobile phase on the retention properties was studied. For this purpose, five 0.001 M phosphate buffer solutions, adjusted to pH 3, 4, 5, 6 and 7, respectively, were prepared. Chromatograms of the "Z" solutions were performed in accordance with the above conditions.

Negligible variations on the retention times were reported in these experiments, so a pH 3 was selected for further ones, because at this pH, the sodium phosphate shows a good buffer capacity and because at lower pH values, the stability of the separation column could be affected.

3.1.3. Effect of the organic solvent in the mobile phase and flow-rate on the retention

In these experiences, the "Z" stock solution was injected into the column and chromatograms were obtained with mobile phases containing different percentages of acetonitrile (15 up to 35%). Obviously, as acetonitrile concentration increased the analysis time decreased, as expected. A 25% acetonitrile was chosen as suitable, according to the peak resolution and run time as well (Fig. 2).

Then, the influence of the flow-rate of the mobile phase was studied. The retention time and chromatographic resolution between peaks decreased when the flow-rate of mobile phase increased. A flow-rate of 1 ml/min was chosen as a compromise between resolution and analysis time.

Finally using the conditions above selected, a satisfactory chromatographic peak resolution was obtained in a short analysis (6 min) as can be seen in Fig. 3.

3.2. Quantitative aspects

3.2.1. Limits of detection and limits of quantification

Limits of detection and quantification (LOD and LOQ) were estimated in accordance with the

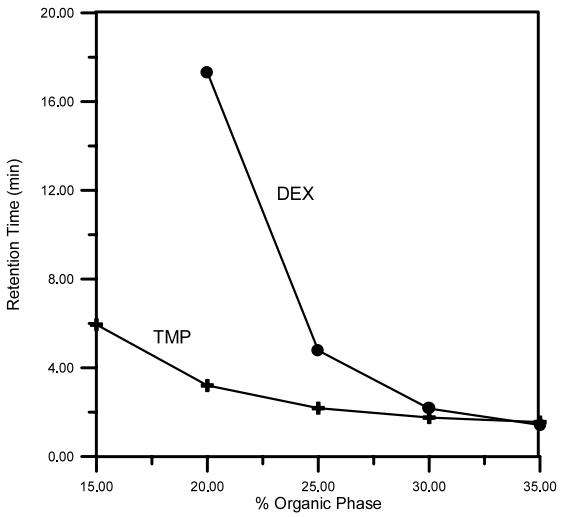


Fig. 2. Influence of the acetonitrile percentage on the retention time.

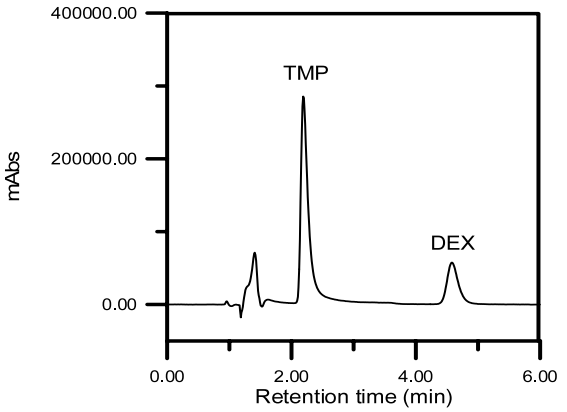


Fig. 3. Chromatogram of a "Z" sample in the optimized conditions, $\lambda = 230$ nm.

baseline noise. The baseline noise was evaluated by recording the detector response over a period of as much as 10 times the peak width. The LOD was obtained as the sample concentration that causes a peak three times as high as the baseline noise level and the LOQ was calculated as being ten times as high as the baseline noise level [32].

Using the parameters above mentioned, LOD and LOQ were estimated to be 70 and 250 μg/l for DEX and 150 and 504 μg/l for TMP.

3.2.2. Linearity range and calibration curves

The linearity of the assay was checked by injecting a set of standards through the chromatographic procedure above described. The calibration curves were obtained for each component by plotting the peak area and the peak height, measured at the maximum absorption wavelengths, 242 and 230 nm for DEX and TMP, respectively, versus concentration. Since the LOQ was estimated around 250 μg/l, the calibration curves started in those concentration levels, but it must be considered the relative composition of the pharmaceuticals to analysis, with regards to determine both components in just one injection.

The results showed that, for DEX and TMP, peak area provided better reproducibility than peak height. Then, peak area was selected for the quantification in all cases.

In Table 1, the equations of the regression linear with the coefficients of determination and the

Table 1
Linear regression calibration curves

Component	Linear regression curve	r^2	Linearity range (mg/l)
DEX	$A = -4274.5(+/-177.8)+31104.5(+/-3128.3)$ Conc. (mg/l)	0.9997	0.3–32
TMP	$A = 66777(+/-2283.7)+157785(+/-40174.1)$ Conc. (mg/l)	0.9982	0.3–32

linear response ranges are presented. In all cases, the intercepts were estimated as negligible by using the Student's test ($\alpha = 0.05$).

3.2.3. Repeatability and intermediate precision

Repeatability was assessed under the chromatographic conditions previously selected by means of 12 replicate injections of a "Z" solution. Intermediate precision was evaluated over 2 days by performing 12 injections each day.

The results showed that the repeatability for every component on each day is satisfactory because the relative standard deviation was found to be about 2.5% for both components. In terms of intermediate precision, the comparison of averages with the Snedecor test [33] did not provide any significant differences between both day' series, for a signification level of 0.05 ($n = 12$). Thus, it can be assumed that our quantitative results are affected by a relative standard deviation of 2.5%.

3.3. Application

3.3.1. Analysis of synthetic mixtures

To test the validity of the proposed method, several synthetic mixtures containing both compounds in different proportions were prepared and analyzed using the chromatographic procedure described. The results are summarized in Table 2. The recoveries (%) obtained show that in the determination of both compounds are very acceptable.

Table 2
Recoveries obtained in the analysis of synthetic mixtures

Composition of mixtures		% Recoveries	
DEX (mg/l)	TMP (mg/l)	DEX	TMP
8	8	99.7	100.7
12	16	100.4	100.4
12	32	98.6	98.6
20	16	101.0	99.9
20	8	98.9	99.9
28	32	97.3	97.9
4	32	96.9	98.7
Statistical parameters			
	X	98.9	99.3
	S	1.53	1.92
	S_m	1.26	1.31

3.3.2. Analysis of pharmaceutical products

The method described was applied to analyzing pharmaceutical formulations containing both of the compounds studied. Thus, we have analyzed four commercial products. The amount of the compound in each sample was calculated by utilizing the standardization and the standard addition method.

The procedure for the preparation of the pharmaceutical preparation is as follows:

3.3.2.1. Quantitative determination by standardization. For all products, once the pharmaceutical mixture homogenized different known aliquots were placed in a 25 ml calibrated flask, adding ethanol (final solution contained 16% ethanol) and deionized water to the mark. A typical sequence contains a number of injections of the calibration and samples solution (calibration 1, sample 1, calibration 2, sample 2, calibration 1), and the response factor was calculated as the average of two consecutive calibration injections.

3.3.2.2. Quantitative determination by standard addition. For all products, once the pharmaceutical mixture homogenized, different known aliquots were placed in 25 ml calibrated flask,

Table 3
Application results (mg/l):recoveries (%)

		Nominal contents (mg/l)	Found contents (mg/l)	:	Recoveries (%)
OTIX	DEX	8	8.23	:	102.93
	TMP	8	7.77	:	97.21
OFTALMOTRIM-DEXA	DEX	8	8.31	:	103.95
	TMP	8	8.09	:	101.14
LIQUIPON	DEX	22.4	22.98	:	102.62
	TMP	–		–	
OFTALMOTRIM	DEX	–		–	
	TMP	14.4	14.21	:	98.71

adding different amounts (2, 4, 6, 8 mg/l) of the compound to determine and proceed as before.

The results are presented in Table 3. Both methods showed similar results and they were performed resulting in agreement of the values obtained.

4. Conclusions

The newly presented HPLC method to determine DEX and TMP proved to be easy to apply in pharmaceuticals because there are no previous sample treatments, apart from the dissolution of the commercials in water and ethanol.

This method proves to be as sensitive, accurate and exact as the derivative spectrophotometric one for this mixture. In this way, by means of derivative ratio spectrum zero-crossing method, the measurement is performed at the specific wavelength previously selected. However, the presence of different excipients in the formulations, might cause a big incidence on the measurement signal. The proposed method, due to the high separation power of HPLC, provides a useful tool for removing the contribution of these interferences, as well as for their detection.

So, it can be concluded that the presented method is convenient for the determination of the studied compounds with appropriate exactness in the quality control of these kinds of pharmaceutical formulations.

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References

[1] J.R. Polinski, R.N. Weinrel, in: M.L. Sears (Ed.), Pharmacology of the Eye, Springer, Heidelberg, 1984, p. 466.
[2] A. Kupferman, M.V. Pratt, K. Suckewer, H.M. Leibowitz, Arch. Ophthalmol. 91 (1974) 373.
[3] J.G. Hardman, L.E. Limbird, P.B. Molinoff, R.W. Ruddon, A. Goodman Gilman, in: A. Goodman Gilman (Ed.), Las Bases Farmacotogicas de la Terapéutica, vol. 2, Mc Graw-Hill, Interamericana de España, Madrid, 1996, p. 1557.
[4] E.V. Michina, N.A. Kalugina, N.G. Filippenko, Otkrytiya Izobret 24 (1991) 149.
[5] P. Sheran, M. O'Keeffe, M.R. Smyth, Analyst 116 (12) (1991) 1365–1368.
[6] P.N. Schil, B.C. Charles, J. Chromatogr. B: Biomed. Appl. 658 (1) (1994) 189–192.
[7] A.I. Santos-Montes, R. Gasco-Lopez, P. Izquierdo Hornillos, Chromatographia 39 (9,10) (1994) 539–542.
[8] E.A. Bunch, J. Assoc. Off. Anal. Chem. 70 (6) (1987) 967–973.
[9] K.-R. Liu, S. Hwei Chem, H. Lug Wu, J. Chromatogr. A 20 (1994).
[10] M. Katayama, Y. Masuda, H. Taniguchi, J. Chromatogr. Biomed. Appl. 612 (1) (1993) 33–39.
[11] J. Ishido, S. Sonezak, M. Yamaguchi, T. Yoshitake, Anal. Sci. 9 (2) (1993) 319–322.
[12] V. Baeyens, E. Varerio, J.L. Veuthey, R. Gurny, J. Chromatogr. 692 (1997) 222–226.
[13] E. Raithk Althoff, J. Bause, H. Neidhart, R.H.H. Neubert, Electrophoresis 19 (16–17) (1998) 1907–2911.

[14] M.T. Ackermans, F.M. Everaerts, J.L. Beckers, J. Chromatogr. 606 (2) (1992) 229–235.
[15] S.K. Wiedmer, H. Siren, M.L. Riekkola, Electrophoresis 18 (10) (1997) 1861–1864.
[16] L. Vomastova, I. MiksiK, Z. Deyl, J. Chromatogr. Biomed. Appl. 681 (1) (1996) 107–113.
[17] M. Alelson, B.L. Shalberg, J. Steroid Biochem. 18 (3) (1983) 313.
[18] L. Hoo, S. Zhao, Zhogguo Yaoxue Zazhi 25 (5) (1990) 246.
[19] H. Zhang, X. Kong, Y. Zhong, Z. Zhu, X. Tang, N. Liu, L. Li, Q. Shao, Taoxue Tongbao 22 (7) (1987) 420.
[20] J.J. Berzas, J.M. Lemus Gallego, G.C. Peñalvo, Fresenius J. Anal. Chem. 342 (1992) 723–728.
[21] J.J. Berzas, J.M. Lemus Gallego, G.C. Peñalvo, Quím. Anal. 13 (1994) 42–46.
[22] C. Yarmitzky, W.F. Smyth, Int. J. Pharm. 75 (20) (1991) 161.
[23] J.J. Bergh, J.C. Breytenbach, J.L. Du Preez, J. Chromatogr. 513 (1990) 392.
[24] L.J. Mulcahey, L.T. Taylor, Anal. Chem. 64 (9) (1992) 981.
[25] S.C. Laizure, C.L. Holden, R.C. Stevens, J. Chromatogr. Biomed. Appl. 93 (1) (1990) 235.
[26] A. Kunkel, H. Wätzig, Arch. Pharm. 332 (5) (1999) 175–178.
[27] A. Kunkel, S. Gunter, H. Waetzig, Electrophoresis 18 (10) (1997) 1882–1889.
[28] H.W. Chen, Z.L. Fang, Anal. Chim. Acta 376 (2) (1998) 209–220.
[29] J. Cao, R.F. Cross, J. Chromatogr. A 695 (2) (1995) 297–308.
[30] K.A. Assi, K.D. Altria, B.J. Clark, J. Pharm. Biomed. Anal. 15 (8) (1997) 1041–1049.
[31] A.B. Wang, F.Y. Li, Y.Z. Fang, Anal. Chim. Acta 386 (3) (1999) 265–269.
[32] K.D. Altria, Y.L. Chanter, J. Chromatogr. 652 (1993) 459.
[33] J.C. Miller, J.N. Miller, Statistics for Analytical Chemistry, Ellis Horwood Limited, London, 1993.