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Short communication

High-performance liquid chromatographic method for the assay of dexamethasone and xylometazoline in nasal drops containing methyl *p*-hydroxybenzoate

Z. Milojevic[a], D. Agbaba[a], S. Eric[a,*], D. Boberic-Borojevic[b], P. Ristic[b], M. Solujic[c]

[a]*Department of Pharmaceutical Chemistry and Drug Analysis, Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, P.O. Box 146, 11 000 Belgrade, Serbia, Yugoslavia*

[b]*Institute of Pharmacy of Serbia, Belgrade, Serbia, Yugoslavia*

[c]*DD 'Sanitarija', 21 000 Novi Sad, Yugoslavia*

Abstract

A rapid and sensitive high-performance liquid chromatographic method has been developed for the determination of dexamethasone sodium phosphate (DSP), xylometazoline hydrochloride (XMC) and methyl *p*-hydroxybenzoate (MHB). An assay of the compounds has been performed on a HPLC system GBC 1210, at controlled room temperature, on a Nucleosil C_8 column (250×3 mm, 5 μm). The mobile phase was acetonitrile–water (35:65, v/v), at a flow-rate of 1 ml min^{-1}. The parameters for validation such as linearity ($r>0.9996$), precision (RSD: 0.51–(1.93%), limit of detection and quantification ($2.032\cdot10^{-4}$ and $4.063\cdot10^{-4}$ mg ml^{-1} for DSP, $9.7\cdot10^{-5}$ and $1.953\cdot10^{-4}$ mg ml^{-1} for XMC, $1.953\cdot10^{-4}$ and $3.096\cdot10^{-4}$ mg ml^{-1} for MHB) have also been reported. The method was applied to the determination of DSP, XMC and MHB in nasal drops. The statistical parameters were found to be satisfactory, with recovery values ranging from 98.69 to 101.60% (RSD: 0.32–1.03%). The method is simple and accurate and therefore suitable for the simultaneous determination of these compounds in dosage form.

Keywords: Pharmaceutical analysis; Dexamethasone sodium phosphate; Xylometazoline hydrochloride; Methyl-*p*-hydroxybenzoate

1. Introduction

Nasal drops containing dexamethasone sodium phosphate (DSP) and xylometazoline hydrochloride (XMC) are very efficient in the therapy of symptoms of allergic rhinitis. Combined application of these compounds is rational, since they act emphasizing their mutual effects [1]. DSP is a glucocorticoid with strong anti-inflammatory and anti-allergic activity. XMC is a selective α_1-agonists and vasoconstrictor, used in topical preparations as long-active nasal decongestant not causing hyperemia as side-effect, in spite of some other decongestants [2]. Methyl-*p*-hydroxybenzoate (MHB) is a good preservative, and primarily protects pharmaceutical preparations against molds.

Different methods have been used for the identification and determination of DSP (colorimetry [3,4], HPTLC [5] or immunoaffinity chromatography [6]) and for XMC (spectrophotometry [7,8] and gas chromatography [9]). Simultaneous determination of DSP and XMC by TLC has also been reported [10].

The most explored technique is HPLC, including

*Corresponding author. Fax: +381-113-972-840.

E-mail address: seric@eunet.yu (S. Eric).

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an assay of dexamethasone and clotrimazole with diode-array detection [11], dexamethasone and betamethasone using gradient elution and diode-array detection [12], dexamethasone and retinyl palmitate [13], dexamethasone, betamethasone and flumethasone pivalate by use of precolumn concentration [14], xylometazoline hydrochloride using external and internal standard methods [15] and ephedrine, naphazoline, oxymethazoline and xylometazoline by gradient elution [16], dexamethasone and xylometazolione [17]. Different preservatives have been used for the preparation of nasal drops with these compounds.

In our previous work, the simultaneous determination of DSP, XMC and MHB as preservative was performed by HPTLC. In this work it was also shown that benzalkonium chloride is not the preservative of choice for such a preparation because of interactions with DSP [18].

To the best of our knowledge, there are no data describing the use of HPLC for the simultaneous determination of DSP, XMC and MHB.

This paper proposes a simple and precise isocratic HPLC method with UV detection for the simultaneous determination of DSP, XMC and MHB. The method is rapid (analyses time of 15 min) and suitable for routine control of these compounds in nasal drops.

2. Experimental

2.1. Chemicals

All chemicals and solvents were of analytical-reagent grade. Acetonitrile and phosphoric acid were obtained from Merck (Darmstadt, Germany). Deionized and distilled water was used.

Standards for DSP [11β,16α-9-fluoro-11,17,21-trihydroxy-16-metylpregna-1,4-diene-3,20-dione-21-disodium phosphate], XMC {2-[[4-(1,1-dimethylethyl)-2,6-dimethylphenyl] methyl]-4,5-dihydro-1H-imidazol hydrochloride} and MHB (methyl ester-4-hydroxybenzoic acid) were obtained from Kimia (UK).

Nasal drops Sanicorten (containing 13.0 mg of DSP, 50.0 mg of XMC and 100.0 mg of MHB per 100 ml) were obtained from Sanitarija (Novi Sad, Yugoslavia).

2.2. Chromatographic equipment and conditions

HPLC analysis was performed on HPLC–GBC 1210 chromatographic system. UV detection was carried out at 225 nm ranged 0.5 AUFS. A GBC Software system was used to collect and integrate the chromatographic data. Nucleosil C_8, 5 μm, 250×3 mm column was used for the separation of these compounds at room temperature. For the analysis, the mobile phase consisted of acetonitrile–water (35:65, v/v) adjusted to pH 2.5 with phosphoric acid, at a flow-rate of 1.0 ml min^{-1}. The injection volume was 20 μl.

2.3. Standard preparations

A stock solution containing 0.104 mg ml^{-1} of DSP, 0.4 mg ml^{-1} of XMC and 0.8 mg ml^{-1} of MHB in mobile phase was used.

2.4. Sample preparations

A 2-ml volume of Sanicorten nasal drops (containing 13.0 mg of DSP, 50 mg of XMC and 100 mg of MHB per 100 ml) was transferred to a 10-ml calibrated flask and diluted with mobile phase to the mark.

2.5. Validation studies

The calibration curves were prepared by diluting the stock solution in the mobile phase to the final concentrations of 0.013, 0.0182, 0.0234, 0.026, 0.0286, 0.0338 and 0.039 mg ml^{-1} for DSP, 0.05, 0.07, 0.09, 0.2, 0.11, 0.13 and 0.15 mg ml^{-1} for XMC and 0.1, 0.14, 0.18, 0.2, 0.22, 0.26 and 0.3 mg ml^{-1} for MHB.

The precision of the method was assessed by determining the intra-day assay RSDs of the analysis ($n=6$) for three concentrations of each compound (0.013, 0.026 and 0.0338 mg ml^{-1} for DSP, 0.05, 0.2 and 0.15 mg ml^{-1} for XMC and 0.1, 0.2 and 0.3 mg

ml^{-1} for MHB). The RSDs for inter-day assay were evaluated by analysis of DSP, XMC and MHB at the same concentrations as it was performed for intra-day assay, repeated for 3 different days.

The accuracy of the HPLC method was proven by the determination of DSP, XMC and MHB from the laboratory-made dosage forms (nasal drops), spiked with 13.0 mg of DSP, 50.0 mg of XMC and 100.0 mg of MHB in 100 ml of purified water with accessory ingredients. The recovery values are expressed as mean values of six repeated analysis.

3. Results and discussion

The HPLC method has been performed on a Nucleosil C_8, 5 μm, 250×3 mm column, using acetonitrile–water (35:65, v/v) adjusted to pH 2.5 with phosphoric acid as the mobile phase, at a flow-rate of 1.0 ml min^{-1}.

The chromatograms obtained are illustrated in Fig. 1. Peaks were well separated and without interference from other compounds in the drops. The retention times were 5.11±0.017 min for DSP, 6.52±0.011 min for MHB and 9.23±0.014 min for XMC.

The validity of the HPLC assay was established through a study of linearity, sensitivity, precision and accuracy. A linear response in peak area ratios was observed over the concentration range 0.013–0.039 mg ml^{-1} for DSP, 0.050–0.15 mg ml^{-1} for XMC and 0.1–0.3 mg ml^{-1} for MHB. The mean of six different calibration graphs yielded the following equations: $y = 25\,509x + 3152$ ($r = 0.9998$) for DSP, $y = 80\,514x - 33\,510$ ($r = 0.9998$) for XMC and $y = 34\,033x + 154\,295$ ($r = 0.9996$) for MHB.

The limits of detection and quantification (LODs and LOQs), were found to be $2.032 \cdot 10^{-4}$ and $4.063 \cdot 10^{-4}$ mg ml^{-1} for DSP, $9.7 \cdot 10^{-5}$ and $1.953 \cdot 10^{-4}$ mg ml^{-1} for XMC, $1.953 \cdot 10^{-4}$ and $3.096 \cdot 10^{-4}$ mg ml^{-1} for MHB, respectively.

The precision of the method was checked with three different concentrations of each compound as intra- and inter-day assay. The method was found to be precise with RSD values within 0.51–1.93% for DSP, 0.68–1.73% for XMC and 1.2–1.9% for MHB (intra-day assay). The RSDs for inter-day assay were 0.92–2.2% for DSP, 0.96–1.85% for XMC and 1.5–2.3% for MHB.

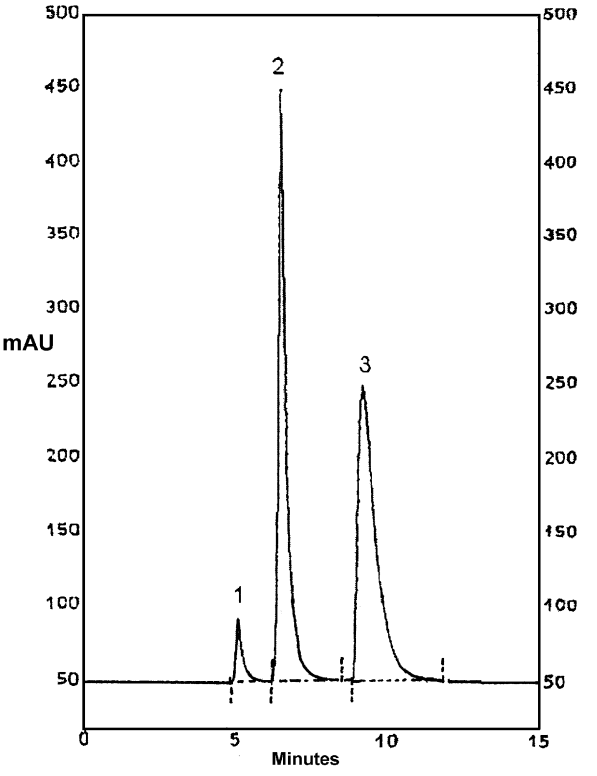

Fig. 1. Chromatograms of DSP (1), MHB (2) and XMC (3) obtained by HPLC. Mobile phase: acetonitrile–water (35:65, v/v) adjusted to pH 2.5 with phosphoric acid; flow-rate 1.0 ml min^{-1}; column: Nucleosil C_8.

The accuracy of the method was established determining the high recovery values for DSP (98.69%), XMC (101.60%) and MHB (100.59%).

Compared with results obtained in our previous work on the simultaneous determination of DSP, XMC and MHB by HPTLC [18], it was shown that the proposed HPLC method provides better linearity, precision of the assay, as well as sensitivity (lower LOD and LOQ values).

The applicability of the HPLC method for the simultaneous determination of DSP, XMC and MHB was verified by the determination of these compounds in Sanicorten nasal drops and the results are given in Table 1. The RSD values obtained, 0.36–1.95%, were found to be satisfactory.

Table 1
Determination of dexamethasone sodium phosphate (DSP), xylometazoline hydrochloride (XMC) and methyl *p*-hydroxybenzoate (MHB) in nasal drops

Compound	Taken (mg)	*n*	Found (mg)	RSD (%)
DSP	13	10	12.69	1.95
XMC	50	10	50.03	1.22
MHB	100	10	94.18	0.36

4. Conclusion

The proposed HPLC method is simple, rapid, and suitable for the routine analysis of DSP, XMC and MHB in nasal drops.

References

[1] K. Parsitt (Ed.), Martindale, 32nd ed., Pharmaceutical Press, London, 1999.
[2] J.T. DiPiro et al. (Eds.), Pharmacotherapy, a Pathophysiologic Approach, Appeleton and Lange, Stamford, 4th ed., 1999.
[3] A.S. Amin, Anal. Lett. 29 (1996) 1527.
[4] L. Ayllon, M. Silva, D. Perez-Bendito, J. Pharm. Sci. 83 (1994) 1135.
[5] J. Hoebus, E. Daneels, E. Roets, J. Hoogmartens, J. Planar Chromatogr. 6 (4) (1993) 269.
[6] H.H.D. Meyer, I. Duersch, Arch. Lebensm. 47 (1996) 22.
[7] M.S. Mahrous, M.E. Abdel-Hamid, H.G. Dabees, Y.A. Beltagy, J. Pharm. Belg. 47 (1992) 135.
[8] S.N. Bhadani, M. Tewarri, A. Agrawal, C. Sekhar, J. Ind. Chem. Soc. 75 (1998) 176.
[9] A. Sioufi, F. Leroux, J.P. Dubois, J. Chromatogr. 487 (1989) 81.
[10] J. Krzek, A. Bielska, Farm. Pol. 41 (1985) 534.
[11] A.M. Di Pietra, V. Andrisano, R. Gotti, V. Cavrini, J. Pharm. Biomed. Anal. 14 (1996) 1191.
[12] A. Santos-Montes, A.I. Gasco-Lopez, R. Izquierdo-Hornillos, Chromatographia 39 (1994) 539.
[13] J.P. Surmann, B. Strahl, C. Hock, Pharmazie 49 (1994) 139.
[14] C. Valenta, H. Janout, J. Liq. Chromatogr. 17 (1994) 1141.
[15] W.F. Ni, O. Jin, Yaowu-Fenxi-Zazhi 16 (1996) 392.
[16] D. De-Orsi, L. Gagliardi, G. Cavazzutti, M.G. Mediati, D. Tonelli, J. Liq. Chromatogr. 18 (1995) 3233.
[17] J.E. Kountourellis, A. Raptouli, Anal. Lett. 21 (1988) 1361.
[18] D. Agbaba, Z. Milojevic, S. Eric, G. Markovic, M. Solujic, M. Aleksic, in: Proceedings of the International Symposium on Planar Separations, New Milestones in TLC, Research Institute for Medicinal Plants, Budakalasz, 2001.