Simultaneous Determination of Ofloxacin, Tetrahydrozoline Hydrochloride, and Prednisolone Acetate by High-Performance Liquid Chromatography

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Abstract

A simple, specific, and precise high-performance liquid chromatographic method has been developed for the simultaneous determination of ofloxacin (OFX), tetrahydrozoline hydrochloride (THC), and prednisolone acetate (PAC) in ophthalmic suspension using propylparaben (POP) as the internal standard. The mobile phase consists of 0.05M phosphate buffer–acetonitrile (65:35, v/v), and the pH is adjusted to 2.7 with orthophosphoric acid. A column containing octadecyl silane chemically bonded to porous silica particles (Waters Spherisorb, 5 µm ODS 1, 4.6 × 150 mm) is used as the stationary phase. The detection is carried out using a variable wavelength UV–vis detector set at 210 nm for OFX and THC and 254 nm for POP (internal standard) and PAC. The solutions are chromatographed at a constant flow rate of 1.2 mL/min. Retention times for OFX, THC, POP, and PAC are approximately 2.5, 4.5, 7.8, and 9.5 min, respectively. The relative retention times are approximately 0.14 min for OFX, 0.35 min for THC, 1.00 min for POP, and 1.22 min for PAC. The linearity range and percent recoveries for OFX, THC, and PAC are 24–120, 4–16, and 16–80 µg/mL and 100.48%, 100.34%, and 100.21%, respectively.

Introduction

Ofloxacin ($C_{18}H_{20}FN_3O_4$) (OFX) is a fluorinated quinolone antibacterial agent used in the treatment of a wide range of infections. It is used topically in the treatment of eye infections. Tetrahydrozoline hydrochloride ($C_{13}H_{16}N_2$•HCl) (THC) is a sympathomimetic agent with α-adrenergic activity. It acts as a local vasoconstrictor. Solutions and suspensions of THC are used as a conjunctival decongestant. Prednisolone acetate ($C_{23}H_{30}O_6$) (PAC) is a glucocorticoid and is used in the topical treatment of allergic and inflammatory conditions of the eyes (1). Several combinations of these drugs are available in the pharmaceutical market for ophthalmic use.

Many analytical procedures have been reported for the determination of OFX, THC, and PAC separately or in combination with other drugs. These include high-performance liquid chromatography (HPLC) employing UV or fluorescence detection, gas chromatography–negative-ion chemical-ionization mass spectrometry, direct or derivative UV spectrophotometry, and microbiology (2–23). OFX and PAC are analyzed by an HPLC procedure and THC is analyzed by a UV spectrophotometric method according to U.S. Pharmacopoeia (USP) (24). These methods are not suitable for their simultaneous determination because of the interferences caused by these components. However, as per the bibliographical revisions performed, no HPLC analytical method applied for the simultaneous determination of these three ingredients containing a combination of the three drugs has been found.

This study describes a rapid, simple, specific, accurate, and precise HPLC method for the simultaneous determination of OFX, THC, and PAC for use in stability studies and quality-control applications associated with these three drugs.

The proposed HPLC method is rapid. The suitability of the analytical procedure is demonstrated by its stability-indicating ability and optimum chromatographic system suitability parameters, which are used for the determination of these medications.

Experimental

Instrumentation

An HPLC system from Shimadzu Corporation (Chromatographic and Spectrophotometric Division, Kyoto, Japan) consisting of an LC-10ATvp solvent delivery module, SCL-10ATvp system controller, SIL-10ATvp automatic sample injector, SPD-10ATvp UV–vis detector, and Waters (Milford, MA) Spherisorb, 5 µm ODS 1 analytical column (4.6 × 150 mm) was used as the stationary phase. Chromatograms were recorded and integrated on a PC installed with Class VP (Shimadzu) chromatographic software.

Reference substances, reagents, and chemicals

OFX was obtained from Zheigiang (China). THC and PAC were obtained from Biophelia (France). Propylparaben (POP) was sup-

plied from Mallinckrodt, Inc. (Derbyshire, U.K.). Potassium dihydrogen phosphate of reagent grade was obtained from Panreac Quimica (Barcelona, Spain) and orthophosphoric acid (85%, w/w) of reagent grade was obtained from BDH Chemicals Ltd. (Poole, U.K.). Acetonitrile of HPLC grade was supplied by Fisher Chemicals (Leicestershire, U.K.). Distilled water was obtained from a Milli-Q system (Millipore, Milford, MA). OFX, THC, PAC, and POP reference standards were obtained from the United States Pharmacopoeia Convention (Rockville, MD).

Chromatographic condition

The mobile phase consisted of a mixture of acetonitrile and 0.05M potassium dihydrogen phosphate buffer at a ratio of 35:65 (v/v), and the pH was adjusted to 2.7 with orthophosphoric acid (85%, w/w). The mobile phase was filtered and degassed using a membrane filter of 0.45-µm porosity under vacuum. A Waters Spherisorb 5-µm ODS 1 analytical column (4.6 × 150 mm) was used as the stationary phase. A constant flow rate of 1.2 mL/min was employed throughout the analysis. The variable UV–vis detector was set initially at 210 nm for OFX and THC detection and then programmed to change to 254 nm at approximately 6.0 min for the detection of POP (internal standard) and PAC. All analyses were performed at room temperature (25°C), and the volume of solution that was injected onto the column was 20 µL.

Diluent

A mixture of acetonitrile and water was prepared at a ratio of 50:50 (v/v). The mixture was sonicated, cooled to room temperature, and filtered through a membrane filter of 0.45-µm porosity.

Samples

The test samples were an ophthalmic suspension with the following compositions per milliliter: 3.0 mg of OFX, 0.4 mg of THC, and 2.0 mg of PAC and excipients quantity sufficient to produce 1.0 mL. Other test samples used were the accelerated stability samples with similar compositions. The samples were treated according to the sample preparation.

Solution preparation

Internal standard solution

A solution containing 0.8 mg of POP per milliliter was prepared by dissolving 80 mg of POP in diluent and diluting it up to volume in a 100-mL volumetric flask.

Standard solution

Standard stock solutions of 1.2 mg OFX per milliliter, 0.4 mg THC per milliliter, and 0.8 mg PAC per milliliter were prepared by dissolving 60 mg of OFX, 20 mg of THC, and 40 mg of PAC in 50 mL of diluent, each separately. Five milliliters of the internal standard solution, 5 mL each of standard stock solutions containing OFX and PAC, and 2 mL of a standard stock solution containing THC were transferred into a 100-mL volumetric flask, and the volume was completed with the diluent to produce concentrations of 60 µg OFX per milliliter, 8 µg THC per milliliter, and 40 µg PAC per milliliter. The solution was mixed, sonicated, cooled to room temperature, filtered, and 20 µL was injected.

Sample solution

Two milliliters of the suspension and 5 mL of the internal standard solution were transferred into a 100-mL volumetric flask and diluted with diluent up to volume. The solution was mixed, sonicated, cooled to room temperature, filtered, and 20 µL was injected.

Degradation of components

The degradation of OFX, THC, and PAC in the mixed standard solution, individual standard solution, and ophthalmic suspension was stimulated by the addition of 0.1N HCl, 0.1N NaOH, and 3% H_2O_2, followed by heating in a water bath at 80°C. The solutions were diluted and examined under similar conditions, but instead using a photodiode-array detector.

Quantitation

Peak areas were recorded for all the peaks, and the peak-area ratios for each component were calculated by dividing the OFX, THC, and PAC areas from the internal standard (POP) area. The respective peak-area ratios were taken into account to quantitate the amounts in milligram per milliliter of the suspension as follows:

$$\frac{R_{sam} \times W}{R_{std} \times 20} \qquad \text{Eq. 1}$$

for OFX and PAC and:

$$\frac{R_{sam} \times W}{R_{std} \times 50} \qquad \text{Eq. 2}$$

for THC, where R_{sam} is the peak-area ratio obtained from OFX, PAC, THC, and the internal standard (POP) in the sample solution; R_{std} is the peak-area ratio obtained from OFX, PAC, THC, and the internal standard (POP) in the standard solution; W is the weight in milligrams of the separate OFX, PAC, THC standards taken to prepare the standard stock solution; and the numerals 20

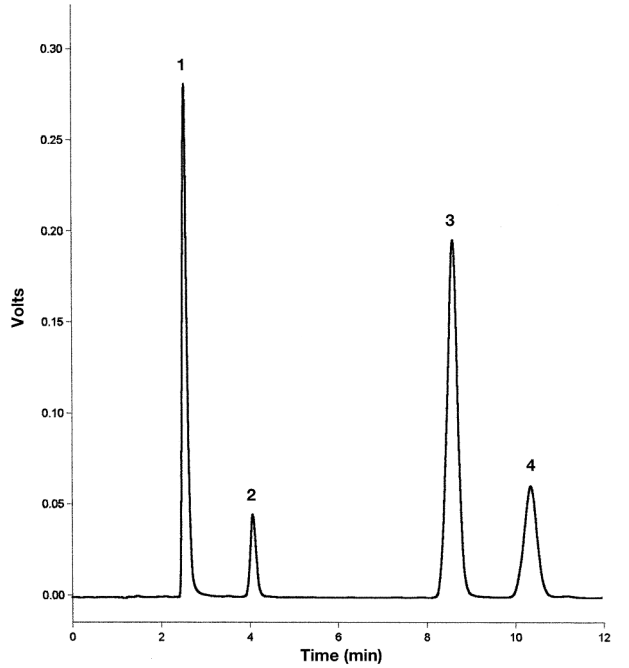

Figure 1. Chromatogram of test sample showing the separated peaks of: OFX, 1; THC, 2; POP, 3; and PAC, 4.

and 50 are the values obtained from the dilution factors of the standard and sample solutions.

Results and Discussion

Chromatography

A mobile phase comprising phosphate buffer and acetonitrile (90:10) for the analysis of OFX in USP24 was used to observe the retention behavior of THC and PAC. This showed significantly less retention time of THC and unresolved peaks of THC and OFX with more tailing. Also, the retention time of PAC was found to be approximately 18 min with a very broad peak shape. An increase of acetonitrile caused good peak shape for PAC, but resulted in a significantly less retention of THC and OFX peaks. Different mobile phases comprising different combinations of 0.05M phosphate buffer, methanol, and acetonitrile tried with different phases bonded to porous silica particles as a stationary phase did not provide sufficient resolution between the three peaks.

The main problem in the development of this method was the separation of OFX and THC with good resolution, long tailing of OFX and THC, and broad peak shape of PAC. It was noted that both a slight increase or decrease in acetonitrile concentration and mobile phase pH had greater effect on chromatography. For example, a slight decrease in acetonitrile concentration resulted in a long retention time and broad peak of PAC, although a very slight improvement in the resolution between OFX and THC was observed. Conversely, a slight increase in its concentration caused a suitable peak for PAC but the resolution of OFX and THC was very much reduced. Also, as the pH of the mobile phase was increased, the resolution between OFX and THC was decreased. This situation was overcome by optimizing the buffer and acetonitrile composition coupled with a pH adjustment towards the acidic side. A mobile phase containing triethylamine together with different combinations of buffer and acetonitrile at varied pHs between 2.7 and 7.0 did not solve this purpose. A mobile phase comprising buffer and acetonitrile in the ratio 60:40 (v/v) and a pH of 2.7 adjusted with orthophosphoric acid (85%, w/w) separated the three peaks with better resolution and good peak shapes, but the quantitative results obtained after analysis were not fairly reproducible, possibly because of the injection volume variation from the automatic sample injector. Therefore, in order to look for a suitable internal standard, different compounds were injected and POP was found to be most suitable with a resolution of 2.0 between POP and PAC peaks. The resolution was further increased to 3.5 by decreasing the acetonitrile concentration from 40% to 35%. The comparatively long retention time of PAC and tailing of the peaks caused by this change was decreased by increasing the flow rate of the mobile phase from 1.0 to 1.2 mL/min. This condition was used to achieve proper retention times, resolution, capacity factor, theoretical plates, and good peak shapes with minimal tailing (Figure 1). This study was limited to carry out a developmental process having a mobile phase pH up to 2.7, because a mobile phase with a lesser pH value (< 2.5)

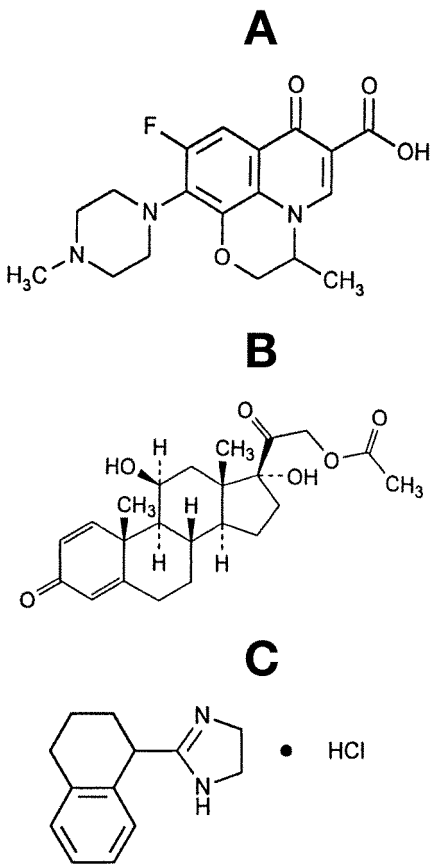

Figure 2. Molecular structure of separated compounds: (A) OFX, (B) PAC, and (C) THC.

Table I. System Suitability Parameters

No.	Component	Area	Tailing factor	Theoretical plates	Capacity factor	Resolution
1	OFX	2100606		1.51 2726		
0.72	–					
	THC	402317	1.34	4872	1.80	7.38
	POP	3317865	1.04	7184	5.08	14.66
	PAC	1223119	1.12	6564	6.20	3.48
2	OFX	2100707	1.52	2732	0.72	–
	THC	400778	1.35	4883	1.79	7.39
	POP	3318804	1.04	7141	5.06	14.62
	PAC	1221899	1.11	6520	6.18	3.48
3	OFX	2107825	1.52	2748	0.72	–
	THC	402527	1.35	4904	5.05	7.41
	POP	3331344	1.04	7174	5.05	14.64
	PAC	1224769	1.12	6556	6.17	3.49
4	OFX	2096409	1.49	2735	0.72	–
	THC	399487	1.32	4889	1.79	7.38
	POP	3315258	1.04	7152	5.05	14.63
	PAC	1221913	1.11	6539	6.17	3.50
5	OFX	2096568	1.50	2739	0.72	–
	THC	400882	1.35	4905	1.79	7.39
	POP	3318505	1.04	7166	5.05	14.63
	PAC	1220837	1.12	6551	6.17	3.50
6	OFX	2096967	1.51	2752	0.72	–
	THC	402544	1.35	4890	1.79	7.40
	POP	3319934	1.04	7219	5.06	14.67

will dissolve the silica, thus creating voids in the column causing changes in the retention time and loss of resolution. A wavelength of 210 nm was selected for OFX and THC to optimize the peak heights in the chromatogram. The detection wavelength of the UV–vis absorbance detector was changed from 210 to 254 nm in order to allow sufficient absorption by POP and PAC. The molecular structure of the three active compounds are shown in Figure 2.

System suitability

System suitability tests were performed to the chromatograms obtained from the standard and sample solutions to check parameters such as column efficiency, tailing factor, capacity factor, and resolution. The results obtained from six replicate injections of the standard solution as representative chromatograms are summarized in Table I.

Linearity

Peak-area ratios versus concentrations in micrograms per milliliter were plotted for OFX, THC, and PAC. OFX, THC, and PAC showed linearity in the range of 24 to 120 µg/mL, 4 to 16 µg/mL, and 16 to 80 µg/mL, respectively. The linear regression equations and the correlation coefficient for these linearities are: $y_{OFX} = 0.0106x - 0.0056$ ($R^2 = 0.9998$); $y_{THC} = 0.0160x - 0.0025$ ($R^2 = 0.9996$); $y_{PAC} = 0.0098x - 0.0094$ ($R^2 = 0.9990$).

Specificity

A blank matrix solution was analyzed, and it was found that there was no interference from other ingredients. The degraded mixed standard, individual standard, and degraded sample solutions were examined under the same chromatographic conditions used for analysis using a photodiode-array detector. There was no interference from the degradant peaks, and the purity of the analyte peaks were found to be 100%. The extent of degradation of the components in the standard solutions were comparatively more than that in the suspension under similar degradation conditions. This difference in the extent of degradation may be ascribed to the resistance of the pharmaceutical formulation to degradation, in which the excipients used may slow down the degradation process.

Table II. Accuracy Data (Analyte Recovery) for OFX

No.	Theoretical amount (mg/mL)	Theoretical (% of target level)	Determined amount (mg/mL)	Determined (% of target level)	Recovered (%)	Bias (%)
1	1.206	40.19	1.213	40.43	100.61	+0.61
2	1.808	60.28	1.830	61.00	101.19	+1.19
3	2.411	80.37	2.428	80.93	100.70	+0.70
4	3.014	100.47	3.009	100.30	99.83	–0.17
5	3.617	120.56	3.604	120.13	99.65	–0.35
6	4.220	140.65	4.265	142.17	101.08	+1.08
7	6.028	200.93	6.048	201.60	100.33	+0.33

Table III. Accuracy Data (Analyte Recovery) for THC

No.	Theoretical amount (mg/mL)	Theoretical (% of target level)	Determined amount (mg/mL)	Determined (% of target level)	Recovered (%)	Bias (%)
1	0.203	50.75	0.201	50.25	99.01	–0.99
2	0.254	63.44	0.258	64.50	101.67	+1.67
3	0.305	76.13	0.306	76.50	100.49	+0.49
4	0.406	101.50	0.412	103.00	101.48	+1.48
5	0.508	126.88	0.503	125.75	99.11	–0.89
6	0.609	152.25	0.605	151.25	99.34	–0.66
7	0.812	203.00	0.822	205.50	101.23	+1.23

Table IV. Accuracy Data (Analyte Recovery) for PAC

No.	Theoretical amount (mg/mL)	Theoretical (% of target level)	Determined amount (mg/mL)	Determined (% of target level)	Recovered (%)	Bias (%)
1	0.803	40.16	0.805	40.25	100.22	+0.22
2	1.205	60.24	1.194	59.70	99.10	–0.90
3	1.606	80.32	1.613	80.65	100.41	+0.41
4	2.008	100.40	2.019	100.95	100.55	+0.55
5	2.410	120.48	2.427	121.35	100.72	+0.72
6	2.811	140.56	2.792	139.60	99.32	–0.68
7	4.016	200.80	4.063	203.15	101.17	+1.17

Accuracy and precision

The accuracy and precision of the proposed HPLC determination were evaluated from the assay result of the components (25).

Accuracy was done by performing the assay of the components calculated from the peak-area responses of different samples by the analyte recovery method. Into blank suspension matrix, OFX and PAC were spiked with 40%, 60%, 80%, 100%, 120%, 140%, and 200% of the target level in the suspension. THC was spiked with 50%, 62.5%, 75%, 100%, 125%, 150%, and 200% of the target level. The mean recovery of the spiked samples was 100.48% for OFX, 100.34% for THC, and 100.21% for PAC (Table II–IV).

Instrumental precision was determined by analyzing the test sample by six replicate determinations, and the relative standard deviations were 0.325% for OFX, 0.218% for THC, and 0.387% for PAC. The relative standard deviations calculated from intraday assay results were 0.703% for OFX, 0.547% for THC, and 0.618% for PAC.

Conclusion

The proposed HPLC method is rapid, direct, accurate, and precise for the simultaneous determination of OFX, THC, and PAC from ophthalmic suspensions. The method can be applied for routine analysis and the quality control of ophthalmic

suspensions or other liquid pharmaceutical preparations containing these drugs, either alone or in combination.

References

1. J.E.F. Reynolds. *Part I, Martindale, The Extra Pharmacopoeia*, 31st ed. Royal Pharmaceutical Society of Great Britain, London, U.K., 1996.
2. D.H. Wright, V.K. Herman, F.N. Konstantinidies, and J.C. Rotschafer. Determination of quinolone antibiotics in growth media by reversed phase high-performance liquid chromatography. *J. Chromatogr. B Biomed. Sci. Appl.* **709(1):** 97–104 (1998).
3. H.J. Kraemer, R. Gehrke, A. Breithaupt, and H. Breithaupt. Simultaneous quantification of cefotaxime, desacetylcefotaxime, ofloxacine and ciprofloxacine in ocular aqueous humour and in plasma by high-performance liquid chromatography. *J. Chromatogr. B Biomed. Sci. Appl.* **700(1-2):** 147–53 (1997).
4. S. Ozyazgan, V. Senses, Z. Ozuner, and A.G. Akkan. Quantification of ofloxacin in biological fluids by high-performance liquid chromatography. *J. Basic Clin. Physiol. Pharmacol.* **8(1-2):** 73–9 (1997).
5. J. Macek and P. Ptacek. Determination of ofloxacin in hyman plasma using high-performance liquid chromatography and fluorescence detection. *J. Chromatogr. B Biomed. Sci. Appl.* **673(2):** 316–19 (1995).
6. D. Fabre, F. Bressolle, J.M. Kinowski, O. Bouvet, F. Paganin, and M. Galtier. A reproducible, simple and sensitive HPLC assay for determination of ofloxacin in plasma and lung tissue. Application in pharmacokinetic studies. *J. Pharm. Biomed. Anal.* **12(11):** 1463–69 (1994).
7. A. Mizuno, T. Uematsu, and M. Nakashima. Simultaneous determination of ofloxacin, norfloxacin and ciprofloxacin in human hair by high-performance liquid chromatography and fluorescence detection. *J. Chromatogr. B Biomed. Appl.* **653(2):** 187–93 (1994).
8. M. Horie, K. Saito, N. Nose, and H. Nakazawa. Simultaneous determination of benofloxacin, denofloxacin, enrofloxacin and ofloxacin in chicken tissues by high-performance liquid chromatography. *J. Chromatogr. B Biomed. Appl.* **653(1):** 69–76 (1994).
9. E.V.L. de Silveira and E.E. Schapoval. Microbiological assay for determination of ofloxacin injection. *J. Pharm. Biomed. Anal.* **27(1-2):** 91–96 (2002).
10. H. Hopkala and D. Kowalczuk. Application of derivative UV-spectrophotometry for the determination of ciprofloxacin, norfloxacin and ofloxacin in tablets. *Acta. Pol. Pharm.* **57(1):** 3–13 (2000).
11. T.G. Altuntas, F. Korkmaz, and D. Nebioglu. Determination of tetrahydrozoline hydrochloride and fluorometholone in pharmaceutical formulations by HPLC and derivative UV spectrophotometry. *Pharmazic* **55(1):** 49–52 (2000).
12. G. Santoni, A. Medica, P. Gratteri, S. Furlanetto, and S. Pinzauti. High-performance liquid chromatographic determination of benzalkonium and naphazoline or tetrahydrozoline in nasal and ophthalmic solutions. *Farmaco* **40(11):** 751–54 (1994).
13. G. Puglisi, S. Sciuto, R. Chillemi, and S. Mangiafico. Simultaneous high-performance liquid chromatographic determination of antazoline phosphate and tetrahydrozoline hydrochloride in ophthalmic solutions. *J. Chromatogr.* **369(1):** 165–70 (1986).
14. G. Andermann and A. Richard. Stability-indicating determination of tetrahydrozoline hydrochloride in ophthalmic solutions by high-performance liquid chromatography. *J. Chromatogr.* **298(1):** 189–92 (1984).
15. J. Bauer and S. Krogh. High-performance liquid chromatographic stability indicating assay for naphazoline and tetrahydrozoline in ophthalmic preparations. *J. Pharm. Sci.* **72(11):** 1347–49 (1983).
16. S.A. Doppenschmitt, B. Scheidel, F. Harrison, and J.P. Surmann. Simultaneous determination of prednisone, prednisolone acetate and hydrocortisone in human serum by high-performance liquid chromatography. *J. Chromatogr. B Biomed. Appl.* **674(2):** 237–46 (1995).
17. S. Lasic, N. Bobarevic, and B. Nikolin. Simultaneous determination of prednisone, prednisolone, cortisol and dexamethasone in plasma by high-performance liquid chromatography. *J. Pharm. Biomed. Anal.* **7(6):** 777–82 (1989).
18. N.R. Scott, J. Chakraborty, and V. Marks. Determination of prednisolone, prednisolone acetate and cortisol in human plasma by high-performance liquid chromatography. *Anal. Biochem.* **108(2):** 87–90 (1980).
19. M. Alvinerie, J.F. Sutra, P. Galtier, G. Houin, and P.L. Toutain. Simultaneous measurement of prednisone, prednisolone and hydrocortisone in plasma by high-performance liquid chromatography. *Anal. Biol. Clin. (Paris)* **48(2):** 87–90 (1990).
20. F. Huber, M. Wiedemann, G. Heinrich, Z. Salama, and H. Jaeger. Development of a high performance liquid chromatographic method for the simultaneous determination of prednisone and prednisolone. *Arzneimittelforschung* **40(8):** 926–31 (1990).
21. J.C. Loo, A.G. Butterfield, J. Moffatt, and N. Jordan. Analysis of prednisolone in plasma by high-performance liquid chromatography. *J. Chromatogr.* **143(3):** 275–80 (1977).
22. P.A. Williams and E.R. Beihl. High-pressure liquid chromatographic determination of corticosteroids in topical pharmaceuticals. *J. Pharm. Sci.* **70(5):** 530–34 (1981).
23. D.G. Watson, J.M. Midgley, and C.N. McGhee. The analysis of corticosteroid acetates by gas chromatography/negative ion chemical ionization mass spectrometry. *Rapid Commun. Mass Spectrom.* **3(1):** 8–10 (1989).
24. *United States Pharmacopeia*. United States Pharmacopeia Convention, Inc., Rockville, MD, 2000. USP 24 NF 19.
25. L.R. Snyder, J.L. Giajch, and J.J. Kirkland. *Practical HPLC Method Development*, 2nd ed. Wiley, New York, NY, 1988.

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