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Simultaneous determination of chondroitin sulfate sodium, allantoin and pyridoxine hydrochloride in pharmaceutical eye drops by an ion-pair high-performance liquid chromatography

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ABSTRACT

An ion-pair high-performance liquid chromatography (HPLC) method has been developed for the simultaneous determination of chondroitin sulfate sodium (CSS), allantoin and pyridoxine hydrochloride (VB_6) in a commercial eye drops dosage form. An Alltima C_{18} column (250 mm × 4.6 mm i.d., 5 μm) was used for the separation at room temperature, with 25 mM ammonium dihydrogen phosphate (containing 0.01% heptanesulfonic acid sodium salt) and acetonitrile (95:5, v/v) as the mobile phase at the flow rate of 0.5 mL min^{-1}. The detection wavelength for CSS, allantoin and VB_6 was 195 nm, 215 nm and 291 nm, respectively. The method showed good linearity for CSS, allantoin and VB_6, with correlation coefficients greater than 0.9996, in the range of 203.96–815.84 mg L^{-1}, 371.16–1488.64 mg L^{-1}, and 23.32–93.28 mg L^{-1}, respectively. The instrumental and method precisions were adequate with all relative standard deviations lower than 2.0%. The accuracy of this method, measured by the recovery of three compounds from spiked placebo solutions, was from 99.01% to 101.92%. The three components, CSS, allantoin and VB_6 were well separated from other ingredients and degradation products. This method is fast, simple, and can be used for direct and simultaneous determination of CSS, allantoin and VB_6 in the pharmaceutical preparation.

1. Introduction

Chondroitin sulfate sodium (CSS) is a sodium salt of a sulfated linear glycosaminoglycan which has disaccharide repeating unit formed by sulfate ester of N-acetylchondrosamine (2-acetamido-2-deoxy-β-D-galactopyronose) and D-glucuronic acid [1]. The chondrosamine moieties in the glycosaminoglycan are monosulfated primarily on position C-4 and less on position C-6 (Fig. 1(A)). These biological polymers act as the flexible connecting matrix in cartilage and tissues [2], and are using as active ingredients in many dietary supplements and pharmaceutical preparations. A photometric titration method using cetylpyridinium chloride has been the standard approach listed in USP for the assay of CSS raw materials and CSS containing tablets [3]. Other methods involving spectrophotometric assay [4,5], high performance capillary electrophoresis (HPCE) assay [6,7], size-exclusion HPLC assay [8], and strong anion-exchange HPLC assay following chondroitinase ABC enzyme digestion [9], have also been reported.

Allantoin (Fig. 1(B)), a purine metabolite, is an astringent and has been reported keratolytic. It is frequently used as a vulnerary to stimulate tissue repair in suppurating wounds, resistant ulcers, acne, seborrhea, cold sores, psoriasis, hemorrhoid, and other anorectal disorders [10,11]. Nowadays, a potentiometric titration assay method is commonly used for the determination of allantoin raw materials in USP [12]. In addition, assays by colorimetry [13,14], hydrophilic interaction chromatography (HILIC) [15], RP-HPLC [16–21], LC–MS/MS [22], capillary electrophoresis (CE) [23,24], and GC–MS [25], have been reported for the determination of allantoin in various types of matrix materials.

Pyridoxine hydrochloride (VB_6) (Fig. 1(C)) is a water-soluble vitamin and is involved primarily in the metabolism of amino acid, carbohydrate, and fat [26]. Many technique, including spectrophotometric, polarographic, fluorimetric, enzymatic, and microbiological approaches have been employed for the determinations of pyridoxine hydrochloride and other water-soluble vitamins. Most of these methods, however, are time-consuming and less accurate. In recent years, micellar electrokinetic capillary chromatography (MEKC) [27], calixarene based potentiometric sensor [28], and HPLC [29–32], have been reported for the analysis of VB_6 from pharmaceutical preparations with improved speed and accuracy. Further, an ion-pairing HPLC method was described [33] in USP.

The purpose of this study was to report, for the first time, a simple HPLC method for the direct and simultaneous determination of CSS, allantoin and VB_6 in a commercial eye drops product.

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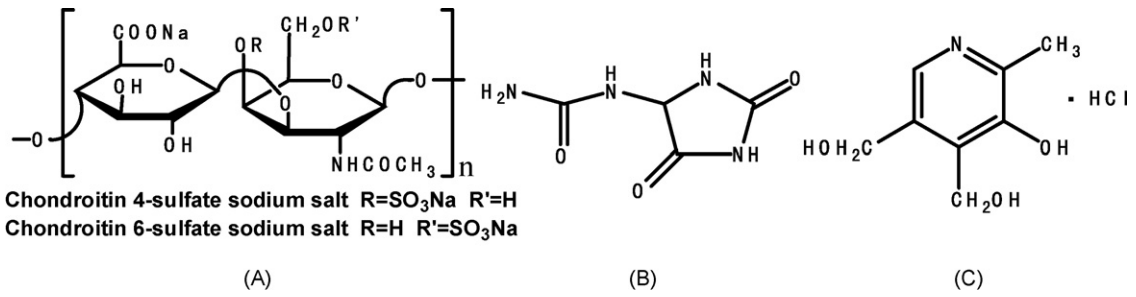

Fig. 1. Chemical structures for disaccharide unit of CSS (A), allantoin (B), and VB_6 (C).

2. Experimental

2.1. Reagents and chemicals

CSS standard raw materials (chondroitin 4-sulfate sodium salt from bovine trachea, lot & filling code: 346459/1 & 33701, HPLC determination purity 93.79%) were purchased from Fluka (Steinheim, Germany). Allantoin and VB_6 reference standards were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Heptanesulfonic acid sodium salt was purchased from Acros (New Jersey, USA). Analytical grade ammonium dihydrogen phosphate, hydrochloric acid, sodium hydroxide, and 30% hydrogen dioxide were obtained from Beijing Reagent Company (Beijing, China). HPLC grade acetonitrile was purchased from Fisher (Fair Lawn, NJ, USA). Redistilled water was prepared by Milli-Q system (Millipore, Bedford, MA, USA). All solvents were filtrated through 0.45-μm PTFE filters (HPLC Technology, Cheshire, UK) before use.

The eye drop sample, compound allantoin vitamin B_6-E and aminoethysulfonic acid eye drops (Eye charm V® eye drops), were made in Japan and obtained commercially. The active ingredients were labeled (per 100 mL) as 100 mg of CSS, 200 mg of allantoin, 10 mg of pyridoxine hydrochloride, 10 mg of tocopherol acetate, and 200 mg of aminoethylsulfonic acid.

2.2. Instrumentation and chromatographic conditions

The study was performed on a Waters 2695 quaternary pump system. A Waters 2696 photodiode array detector and an Empore professional® software were used for data acquisition and processing. The chromatographic separations were carried out on an Alltima C_{18} column (250 mm × 4.6 mm i.d., 5 μm, 100 Å, Alltech Associates Inc., Deerfield, IL, USA). The mobile phase composed of 25 mM ammonium dihydrogen phosphate (containing 0.01% heptanesulfonic acid sodium salt) and acetonitrile (95:5, v/v) was delivered at a flow rate of 0.5 mL min^{-1}. The selected detection wavelengths for CSS, allantoin and VB_6 were 195 nm, 215 nm, and 291 nm, respectively. The injection volume was 10 μL. All analysis was conducted at room temperature.

2.3. Preparation of standard solutions

A stock solution containing 2039.6 mg L^{-1} CSS, 3721.6 mg L^{-1} allantoin, and 233.2 mg L^{-1} VB_6 was prepared by dissolving accurately weighed amounts of standards in the mobile phase. Sequential dilutions were then made using the mobile phase, to reach final concentrations of 203.96 mg L^{-1}, 305.94 mg L^{-1}, 407.92 mg L^{-1}, 509.90 mg L^{-1}, and 815.84 mg L^{-1} for CSS, 371.16 mg L^{-1}, 558.24 mg L^{-1}, 744.32 mg L^{-1}, 930.40 mg L^{-1}, and 1488.64 mg L^{-1} for allantoin, and 23.32 mg L^{-1}, 34.98 mg L^{-1}, 46.64 mg L^{-1}, 58.30 mg L^{-1}, and 93.28 mg L^{-1} for VB_6. The mid-point concentration in these dilutions was used as working standard solution for system suitability studies.

2.4. Preparation of analytical samples

2 mL of the Eye charm V® eye drops were transferred into a 5 mL volumetric flask, made up to 5 mL with mobile phase and vortexed for 5 min. The resulting solutions (sample solutions) were then analyzed by HPLC after filtration through 0.20-μm PTFE membrane syringe filter (Anachem, Cheshire, UK).

2.5. Validation procedure

The developed method was validated for system suitability, specificity, linearity, precision, accuracy and LOD, following ICH recommendations [34,35].

2.5.1. System suitability

The key system suitability parameters, including theoretical plates and asymmetry factors for chromatographic peaks of CSS, allantoin, and VB_6 were calculated as European Pharmacopoeia [36] described.

2.5.2. Specificity

To assess the method specificity, a reconstituted eye drop placebo without CSS, allantoin, and VB_6 was prepared. The placebo solution was prepared using the same procedure as in preparing for the analytical samples (Section 2.4), and then subjected to HPLC analysis to evaluate the potential interferences from other ingredients (i.e. aminoethylsulfonic acid, tocopherol acetate, isoosmotic adjusting agent, etc.).

Forced degradation studies of CSS standard, allantoin standard, VB_6 standard, reconstituted placebo, and eye drop sample, under different stress conditions (heat, light, oxidation, acid and base), were conducted to evaluate the potential interferences of degradation products.

For preparing heat degradation products, 5 mL of placebo solution, 5 mL of analytical sample solution, 5 mL of CSS standard solution (400 mg L^{-1}, approximately), 5 mL of allantoin standard solution (800 mg L^{-1}, approximately), and 5 mL of VB_6 standard solution (40 mg L^{-1}, approximately), were heated at 80 °C for 24 h in dark, and then cooled to room temperature. To study light induced degradation products, these solutions were accumulatively exposed to directed sunlight for more than 48 h.

For preparing oxidation resulted degradation products, 1 mL of 9.0% hydrogen peroxide (v/v) was added to 2 mL of reconstituted placebo and 2 mL of eye drop sample, while 1 mL of 3.0% hydrogen peroxide (v/v) was added to 20 mg of CSS standard, 40 mg of allantoin standard, and 2 mg of VB_6 standard. After stored in dark for more than 24 h, the degraded placebo and eye drop sample were transferred into a 5 mL volumetric flask and brought to volume with the mobile phase, while the degraded standards were dissolved

and transferred into a 50 mL volumetric flask and also brought to volume with the mobile phase.

To study acid and base induced degradation products, 2 mL of 1 M HCl solutions or 2 mL of 1 M NaOH solutions were separately added to 2 mL of reconstituted placebo, 2 mL of eye drop sample, 20 mg of CSS standard, 40 mg of allantoin standard, and 2 mg of VB_6 standard. These solutions were then refluxed and heated at 80 °C in a water bath for 2 h in dark, and cooled to room temperature. Each degraded sample was then neutralized with NaOH or HCl, and transferred into a 5 mL volumetric flask (placebo and eye drop sample) or a 50 mL volumetric flask (standards), and brought to volume with mobile phase.

2.5.3. Linearity

Linearity of the method was evaluated at five concentrations level, covering about 50%, 75%, 100%, 125%, and 200% of the targeted assay concentration. Injections were made in triplicate and the resulted peak areas were inputted into a Microsoft Excel spreadsheet to plot the calibration curves.

2.5.4. Precision

The instrumental precision was investigated by analyzing six consecutive injections of low, middle and high concentration standard solutions on the same day and on 6 different days. The %R.S.D. values of peak areas obtained on the first day were used to evaluate the intra-day precision, and the %R.S.D. values of average peak areas for 6 other days were used to evaluate the inter-day precision.

For the evaluation of method precision (repeatability and intermediate precision), six independent sample solutions were prepared and analyzed on 6 different days. The intra-day %R.S.D. values of the assay results in the first day were used to examine method repeatability, and the inter-day %R.S.D. values of six average assay results were used to evaluate intermediate precision.

2.5.5. Accuracy

To assess accuracy, a freshly prepared placebo was spiked with various amounts of combined stock solutions, and diluted with the mobile phase to concentrations at about 75%, 100%, and 125% of the targeted concentration. Each spiked solution was injected in triplicate and the peak areas were used to calculated mean and %R.S.D. values, and compared with those obtained with standard solutions.

2.5.6. Limit of detection

The LOD was determined by injecting progressively low concentration of standard solutions for nine replicates that generating a S/N ratio of 3.

3. Results and discussion

3.1. Method development

The aim of this study was to develop a simple HPLC method for the simultaneous analysis of CSS, allantoin, and VB_6 in the presence of other ingredients and forced degradation products. Due to the extreme polar nature, these compounds were unlikely to retain on reversed phase C_{18} HPLC column, and base-line separations were difficult to achieve. Extending the retention times of the testing compounds could improve the resolution, and the selection of a suitable buffer solution would be an effective way to reach this purpose. In this study, relatively bigger retention factors and good resolutions were obtained when performed with a mobile phase consisting of ammonium dihydrogen phosphate solution and acetonitrile. However, unacceptable system suitability parameters for VB_6 were obtained, with theoretical plates of less than 2000 and asymmetry factors bigger than 2.0. To improve the system suitability for VB_6, an ion-pair reagent, heptanesulfonic acid, was introduced into the phosphate buffer.

To optimize the separation, phosphate buffer at 10 mM, 25 mM, and 50 mM concentrations mixed with acetonitrile at ratios of 98:2, 95:5, and 90:10, has been tested. Effects of flow rate (0.5 mL min^{-1}, 1.0 mL min^{-1}, and 1.5 mL min^{-1}) and temperature (25 °C, 30 °C, and 40 °C) were also investigated systematically. The conclusions were that buffer concentration, flow rate, and temperature have no significant effects on separations.

The concentration of heptanesulfonic acid in buffer solutions, however, implies significant effects on the retention time of VB_6. Longer retention time was observed at higher concentrations. It was therefore lowest possible concentration of heptanesulfonic acid, 0.01%, was selected in final experimental conditions.

Further, effects of pH value (3.0, 3.5, 4.0, 5.0, 6.0, and 7.0) of the buffer solution have been studied. Results indicated that at low pH (3.0 and 3.5) the signal of CSS decreases, while at high pH (6.0 and 7.0) the signal of VB_6 decreases. At middle pH, adequate signal intensities of all components were observed, therefore, the buffer solution without pH adjust (pH 4.3) was used for the following experiments.

Usually the UV detection window was set at the wavelength of maximum UV signals produced by a component. Both CSS and allantoin showed maximum absorbance at 195 nm, the linearity of allantoin, however, was unacceptable ($r < 0.9900$) when detected at this window, thus, it was set at 215 nm for allantoin as reported in literatures [19,20]. The UV spectrums of VB_6 showed significant absorbance at 291 nm, therefore, the detection wavelength for VB_6 was set at 291 nm accordingly.

Table 1
Validation of the method regarding system suitability, linearity, and limits of detection.

Analytical parameter	CSS	Allantoin	VB_6
Detection wavelength (nm)	195	215	291
Theoretical plate[a] (mean ± SD)	3,649 ± 49	5,371 ± 23	4,707 ± 94
Asymmetry factor[a] (mean ± SD)	1.212 ± 0.008	1.043 ± 0.003	1.301 ± 0.015
Linear range (mg L^{-1})	203.96 – 815.84	371.16 – 1,488.64	23.32 – 93.28
Linear equation	$Y = 11{,}024X + 16{,}575$	$Y = 12{,}391X - 4{,}542$	$Y = 35{,}771X - 101{,}348$
Confidence limits for slopes[b]	10,840–11,208	12,270–12,512	35,199–36,343
Confidence limits for intercepts[b]	−74,631 to 107,781	−114,455 to 105,371	−137,678 to −69,018
Coefficient of correlation (r)	0.9996	0.9999	0.9997
Limit of detection (mg L^{-1})	0.204	0.092	0.233

[a] Working standard solution was performed for five replicates.
[b] 95% confidence interval.

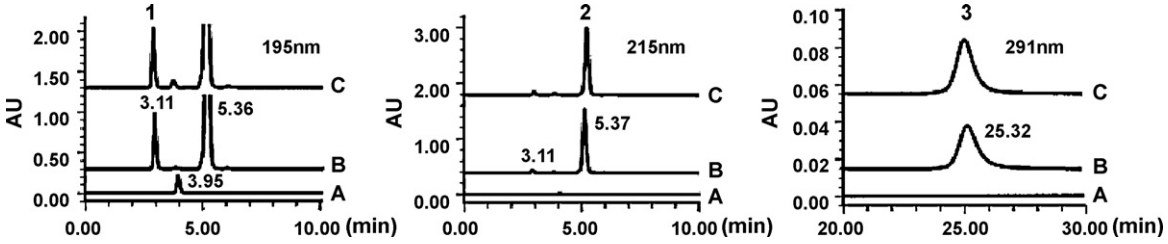

Fig. 2. Chromatograms for placebo (A), combined standards (B), and the analytical sample (C), detected at 195 nm, 215 nm, and 291 nm.

3.2. Method validation

3.2.1. System suitability

System suitability test is for verifying that the system is adequate for the analysis to be performed. In this paper, theoretical plates and asymmetry factors for CSS, allantoin, and VB_6 were evaluated. All those parameters, reported in Table 1, met the acceptable criteria of the FDA CDER guidance [37].

3.2.2. Linearity

In this paper, five concentration levels were used to study the linear dynamic range of the method. Results were reported in Table 1 with great correlation ($r\hat{0}$.9996–0.9999) of UV responses of all three components within 50–200% of the targeted concentrations.

3.2.3. Limit of detection

The limits of detection for CSS, allantoin and VB_6 in this method were showed in Table 1, briefly, 0.092–0.233 mg L^{-1} for all three compounds.

3.2.4. Specificity

The representative chromatograms of the placebo (Fig. 2(A)), combined standards (Fig. 2(B)), and the analytical sample (Fig. 2(C)), showed that additives or matrices were well separated from the three major components, CSS, allantoin, and VB_6.

Further evaluation of selectivity was conducted by monitoring the separation of degradation products from the components of interest. Samples containing CSS, allantoin, VB_6, placebo, and the eye drop were subjected to stress conditions under heat, light, hydrogen peroxide, acid and base, and analyzed at the same chromatographic conditions. No significant degradation products were observed from placebo under any stress conditions; CSS degraded under both acidic and basic conditions, its degradation products (Fig. 3(A)–(D)) were well resolved from other analytes; Allantoin was found to degrade under basic conditions (Fig. 3(E) and (F)), and VB_6 produced degradation products when treated with sunlight (Fig. 3(G) and (H)), however, these species had no interferences with the determination of CSS and allantoin. Moreover, all of the above-mentioned degradation products did not show chromatographic peaks at 291 nm (data not shown); their interferences to VB_6 peak were also ignored.

The eye drop sample was also investigated under stress conditions; no additional degradation products were observed.

3.2.5. Precision

The instrumental and method precisions were evaluated at three concentration levels covering 50%, 100%, and 200% of target concentrations for 6 days. Results obtained were shown in Table 2. In all cases, the %R.S.D. values were less than 2%.

3.2.6. Accuracy

Results obtained were shown in Table 3. The values demonstrated that the method was accurate within the desired ranges.

3.2.7. Analysis of a commercial pharmaceutical preparation

The validated method was successfully applied for the simultaneous determination of CSS, allantoin and VB_6 in four batches of commercial eye drops (Eye charm V® eye drops). The representative chromatograms were shown in Fig. 2(C). Results were showed in Table 4.

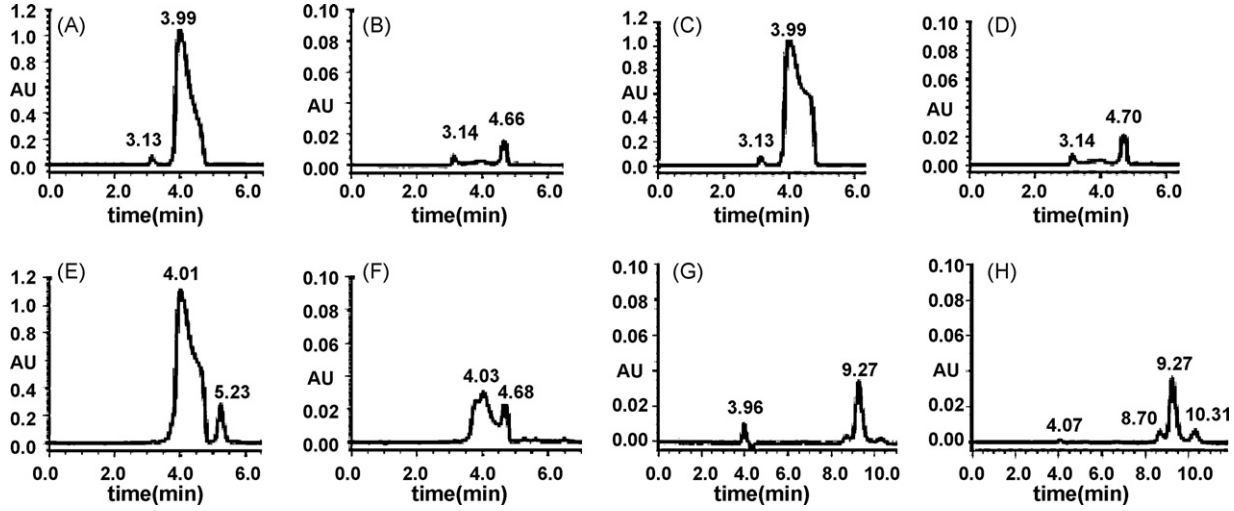

Fig. 3. Chromatograms for forcedly degraded sample solutions: (A) CSS, acidic condition, 195 nm; (B) CSS, acidic condition, 215 nm; (C) CSS, basic condition, 195 nm; (D) CSS, basic condition, 215 nm; (E) Allantoin, basic condition, 195 nm; (F) Allantoin, basic condition, 215 nm; (G) VB_6, sunlight treatment, 195 nm; (H) VB_6, sunlight treatment, 215 nm.

Table 2
Precision results for CSS, allantoin and VB_6.

Analyte	Instrumental precision (%R.S.D.)			Method precision (%R.S.D.)	
	% of target concentration[a]	Intra-day ($n=6$)	Inter-day ($n=6$)	Repeatability ($n=6$)	Intermediate precision ($n=6$)
CSS	50	0.42	1.08	1.01	1.57
	100	0.43	0.89		
	200	0.14	0.50		
Allantoin	50	0.21	0.83	0.79	0.90
	100	0.18	0.50		
	200	0.12	0.36		
VB_6	50	0.67	1.31	1.26	1.92
	100	0.70	1.03		
	200	0.54	0.88		

[a] 100% of target concentration is equivalent to 400 mg L^{-1} of CSS, 800 mg L^{-1} of allantoin, and 40 mg L^{-1} of VB_6.

Table 3
Accuracy: % recovery data for CSS, allantoin and VB_6.

% of target concentration [a]	CSS% recovery	Allantoin% recovery	VB_6% recovery
75	99.93 (0.07)	99.01 (0.14)	99.68 (1.51)
100	100.01 (0.25)	99.96 (0.25)	100.49 (0.47)
125	100.38 (0.07)	101.92 (0.13)	100.66 (0.57)
Average % recovery	100.11	100.30	100.28

[a] 100% of target concentration is equivalent to 400 mg L^{-1} of CSS, 800 mg L^{-1} of allantoin, and 40 mg L^{-1} of VB_6. The figures in parenthesis represent %R.S.D. values for three replicates ($n=3$).

Table 4
The contents of CSS, allantoin and VB_6 in the eye drop samples.

Batches	CSS% of label claim[a]	Allantoin% of label claim[a]	VB_6% of label claim[a]
SKC	100.02 (1.37)	95.15 (1.48)	95.56 (1.70)
TCA	99.29 (0.53)	92.53 (0.39)	91.65 (1.86)
RCA	109.26 (0.34)	101.13 (0.38)	105.56 (1.55)
SEB	101.51 (0.57)	94.72 (0.76)	97.18 (0.83)

[a] 100% of label claim is equivalent to 1000 mg L^{-1} of CSS, 2000 mg L^{-1} of allantoin, and 100 mg L^{-1} of VB_6. The figures in parenthesis represent %R.S.D. values for three replicates ($n=3$).

4. Conclusions

Separation of CSS, allantoin and VB_6 in a commercial eye drops by HPLC has been very challenging owing to their polar natures and poor retentions on reversed phase C_{18} HPLC column. We developed an ion-pair HPLC approach with good system suitability, sensitivity, linearity, specificity, precision, and accuracy. CSS, allantoin and VB_6 were not only separated from each other, but from other ingredients and potential degradation products. This method appears to be reliable and convenient for direct and simultaneous determinations of CSS, allantoin and VB_6 in pharmaceutical preparations.

References

[1] The Merck Index, 13th ed., MERCK & CO. INC., Whitehouse Station, NJ, 2001, p. 383.
[2] F. Fawthrop, R. Yaqub, C. Belcher, M. Bayliss, J. Ledingham, M. Doherty, Ann. Rheum. Dis. 56 (1997) 119–122.
[3] USP29-NF24, United States Pharmacopeial Convention Inc., Rockville, MD, 2006, pp. 2306–2308.
[4] T. Bitter, H.M. Muir, Anal. Biochem. 4 (1962) 330–334.
[5] M. Okamoto, S. Mori, M. Ishimaru, H. Tohge, Y. Nakata, H. Endo, Life Sci. 60 (1997) 1811–1819.
[6] T. Oguma, H. Toyoda, T. Toida, T. Imanari, Biomed. Chromatogr. 15 (1995) 918–925.
[7] F. Lamari, A. Theocharis, A. Hjerpe, N.K. Karamanoe, J. Chromatogr. B 730 (1999) 129–133.
[8] D.W. Choi, M.J. Kim, H.S. Kim, S.H. Chang, G.S. Jung, K.Y. Shin, S.Y. Chang, J. Pham. Biomed. Anal. 31 (2003) 1229–1236.
[9] J.-S. Sim, G. Jun, T. Toida, S.Y. Cho, D.W. Choi, S.-Y. Chang, R.J. Linhardt, Y.S. Kim, J. Chromatogr. B 818 (2005) 133–139.
[10] Martindale, The Complete Drug Reference, vol. 1, 34th ed., The Pharmaceutical Press, London, U.K., 2005, p. 1141.
[11] Remington, The Science and Practice of Pharmacy, vol. 2, 21th ed., Lippincott, Williams, and Wilkins, Philadelphia, PA, 2005, p. 1290.
[12] USP29-NF24, United States Pharmacopeial Convention Inc., Rockville, MD, 2006, p. 74.
[13] H. Muratsubaki, K. Satake, K. Enomoto, Anal. Biochem. 359 (2006) 161–166.
[14] H. Muratsubaki, K. Enomoto, A. Soejima, K. Satake, Anal. Biochem. 378 (2008) 65–70.
[15] P. Dallet, L. Labat, E. Kummer, J.P. Dubost, J. Chromatogr. B 742 (2000) 447–452.
[16] S.K. George, M.T. Dipu, U.R. Mehra, P. Singh, A.K. Verma, J.S. Ramgaokar, J. Chromatogr. B 832 (2006) 134–137.
[17] M. Czauderna, J. Kowalczyk, J. Chromatogr. B 744 (2000) 129–138.
[18] G. Haghi, R. Arshi, A. Safaei, J. Agric. Food Chem. 56 (2008) 1205–1209.
[19] M. Czauderna, J. Kowalczyk, J. Skomial, K.M. Niedzwiedzka, J. Anim. Feed Sci. 13 (Suppl 2) (2004) 67–70.
[20] K.J. Shingfield, N.W. Offer, J. Chromatogr. B 706 (1998) 342–346.
[21] P. Garcia del Moral, M.T. Diez, J.A. Resines, I.G. Bravo, M.J. Arin, J. Liq. Chromatogr. Rel. Technol. 26 (17) (2003) 2961–2968.
[22] A. Berthemy, J. Newton, D. Wu, D. Buhrman, J. Pham. Biomed. Anal. 19 (1999) 429–434.
[23] E. Causse, A. Pradelles, B. Dirat, A. Negre-Salvayre, R. Salvayre, F. Couderc, Electrophoresis 28 (2007) 381–387.
[24] D. Kattygnarath, N. Mounier, I. Madelaine Chambrin, B. Gourmel, T. Le Bricon, C. Gisselbrecht, P. Faure, P. Houze, Clin. Biochem. 39 (2006) 86–90.
[25] D.V. Pavitt, S. de Fonseka, N. Al Khalaf, J.M. Cam, D.A. Reaveley, Clin. Chim. Acta 318 (2002) 63–70.
[26] Martindale, The Extra Pharmacoeia, 31th ed., Royal Pharmaceutical Society of Great Britain, London, U.K., 1996, p. 1384.
[27] USP29-NF24, United States Pharmacopeial Convention Inc., Rockville, MD, 2006, pp. 1869–1871.
[28] E. Nemutlu, M. Celebier, B. Uyar, S. Altinoz, J. Chromatogr. B 854 (2007) 35–42.
[29] V.D. Vaze, A.K. Srivastava, J. Pham. Biomed. Anal. 47 (2008) 177–182.
[30] R. Gatti, M.G. Gioia, V. Cavrini, Anal. Chim. Acta 512 (2004) 85–91.
[31] M.L. MarszaИ, A. Lebiedzińska, W. Czarnowski, P. Szefer, J. Chromatogr. B 1094 (2005) 91–98.
[32] A. EL-Gindy, F. EL-Yazby, A. Mostafa, M.M. Maher, J. Pham. Biomed. Anal. 35 (2004) 703–713.
[33] C.K. Markopoulou, K.A. Kagkadis, J.E. Koundourellis, J. Pham. Biomed. Anal. 30 (2002) 1403–1410.
[34] ICH-Q2A, Text on Validation of Analytical Procedures, March 1995.
[35] ICH-Q2B, Validation of Analytical Procedures: Methodology, November 1996.
[36] European Pharmacopoeia, 5th ed., Council of Europe, Strasbourg, 2005, pp. 69–72.
[37] Center for Drug Evaluation and Research, U.S. Food and Drug Administration, Reviewer Guidance, Validation of Chromatographic Methods: FDA, Rockville, MD, November 1994.