High-Performance Liquid Chromatographic Quantitative Determination of Amcinonide and Benzyl Alcohol in Pharmaceutical Preparations

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Abstract: This paper describes a simple, precise, and validated high-performance liquid chromatographic method for the simultaneous quantitative determination of amcinonide and benzyl alcohol in pharmaceutical preparations. The separation was achieved using a Zorbax reversed-phase column (5µm, 250x4.6mm) at room temperature with an isocratic mixture of deionized water and acetonitrile (70:30 v/v) at a flow rate of 1.5ml/min and detection at 254 nm. The limits of quantification for amcinonide and benzyl alcohol were found to be 4-30 µg/ml and 80-600 µg/ml, respectively. The validity of the method was evaluated in terms of linear regression analysis, precision, specificity, accuracy and ruggedness. The developed methodology is an economic, time-saving, straight forward and precise assay for the determination of amcinonide and benzyl alcohol in pharmaceutical preparations. It can be readily utilized for quality assurance and R&D laboratories of pharmaceutical and cosmetic industry.

INTRODUCTION

Amcinonide is a corticosteroid applied as an anti-inflammatory agent [1]. It is used in different pharmaceutical preparations such as film-forming creams, lotions and ointments for the treatment of skin disorders [2]. It exerts great influence on the structure and function of tail epidermis of yellow and black mice [3]. Amcinonide is used in pharmaceutical preparations alone or in combination with other steroids such as betamethasone valerate, hydrocortisone-17-butyrate or flucinonide [4]. It is also mixed with some fungicides, used for mouthwashes [5]. Steroids have been used in pharmaceutical preparation since long to treat rheumatic, neoplastic and other disorders [6].

Amcinonide creams and ointments contain some preservative such as benzyl alcohol. Usually quantitative estimation of amcinonide and benzyl alcohol is done by two separate analytical methods. A high performance liquid chromatographic method with gradient elution of mobile phase is described for the quantification of amcinonide creams and of its raw materials [7]. A spectrophotometric method is also known for the determination of amcinonide in different pharmaceutical dosage forms which involves a lengthy extraction process followed by adsorption chromatography on silica gel as stationary phase and by spectrophotometric detection [8].

Application of thin layer chromatography to determine amcinonide in ointments and creams has been reported by several groups what proved to be a non-specific approach [9,10]. Simultaneous determination of hydrocortisone and benzyl alcohol in pharmaceutical formulations by reversed phase chromatography was also reported [11].

Benzyl alcohol is used as an antimicrobial preservative in pharmaceutical and cosmetic products and its concentration should not exceed limit values for each formulation type while it can produce fatal toxic effects, allergies and various other effects on the nervous system [12,13]. Benzyl alcohol is quantitatively estimated in raw material and pharmaceutical preparations by gas chromatography, derivative spectrophotometry, HPLC, amperometric enzyme electrode, polarography and capillary electrokinetic chromatography [13]. A high performance liquid chromatographic method for simultaneous determination of bifonazole and benzyl alcohol in pharmaceutical formulations is also reported [13]. In another report a HPLC method for simultaneous determination of benzyl alcohol and deslorelin [14] is described. To the best of our knowledge no analytical method is available for the simultaneous determination of amcinonide and benzyl alcohol in pharmaceutical preparations. Therefore, it was considered necessary to develop a HPLC method for the quantification of these compounds in pharmaceutical samples.

In the context of developing new quantitative determination procedures for natural constituents and drugs in body fluids using HPLC and capillary electrophoresis techniques we report various methods [15].

We wish to describe herein a simple, rapid, economical, and accurate method of analysis using reversed-phase HPLC (RP-HPLC) to simultaneously determine amcinonide and

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benzyl alcohol in pharmaceutical preparations and also the validation of this newly developed method. To the best of our knowledge this is for the first example of simultaneously determination of this type.

MATERIALS AND METHODS

HPLC grade acetonitrile and water (E. Merck, Darmstadt, Germany) were used for the HPLC analysis. Amcinonide and benzyl alcohol were USP reference standards.

Apparatus and Chromatographic Conditions

A Shimadzu 6 A system (Shimadzu, Tokyo, Japan), comprising a LC-6A pump, a SCL-6A system controller, a SIL-6A auto-injector, a SPD-6A, an UV-detector and C-R6A chromatopac was used. The column configuration was a Zorbax ODS reversed-phase column (5µm, 250x4.6 mm) with a RP8 RPGU (Shimadzu, Tokyo, Japan), guard column (10 µm, 10x4.6 mm). An aliquot of 20 µl was chromatographed.

The detection wavelength was set at 240 nm. The flow rate was 1.5 ml/min and the separation was performed at ambient temperature. The mobile phase consisted of a degassed filtered mixture of acetonitrile and water (70:30 % v/v).

Preparation of Standard Solution

Accurately weighed 20 mg of amcinonide and 400 mg of benzyl alcohol were transferred into a 100 ml volumetric flask dissolved in 50 ml of mobile phase with sonication for 5 minutes and made up to volume with mobile phase. An aliquot of 5 ml of this solution was transferred into a 50 ml volumetric flask and diluted to volume with a mixture of acetonitrile water (70:30; v/v). The solution was filtered through a 0.45 µm membrane filter before injection.

Sample preparation

A pharmaceutical sample containing amcinonide and benzyl alcohol, equivalent to 2 mg and 40 mg, respectively, was weighed and transferred into a 100 ml volumetric flask. The contents of the flask were dispersed in 30 ml of a mixture of acetonitrile and water (70:30; v/v) with heating on a water bath at a temperature not exceeding 50 $^{\circ}$C for 5 minutes. While the flask was hot, 50 ml of the same solvent mixture was added and the materials were dissolved by sonication for 5 minutes and made up to volume with the same solvent mixture. The solution was filtered through a Whatman No. 541 filter paper and then through a 0.45 µm membrane filter before injection.

RESULTS AND DISCUSSIONS

Standard solutions of amcinonide and benzyl alcohol containing 50, 60, 70, 80, 90, 100, 120, 130, 140 and 150% of theoretical assay concentration (20 mg/ml and 400 mg/ml) were examined by the proposed HPLC method. A linear relation was observed between peak area and concentration. Fig. (**1**) shows a typical chromatogram.

Regression analysis of the calibration data (*n* = 3) was carried out and the value of slope, intercept and correlation co-efficient for each calibration curve were calculated (Table **1**).
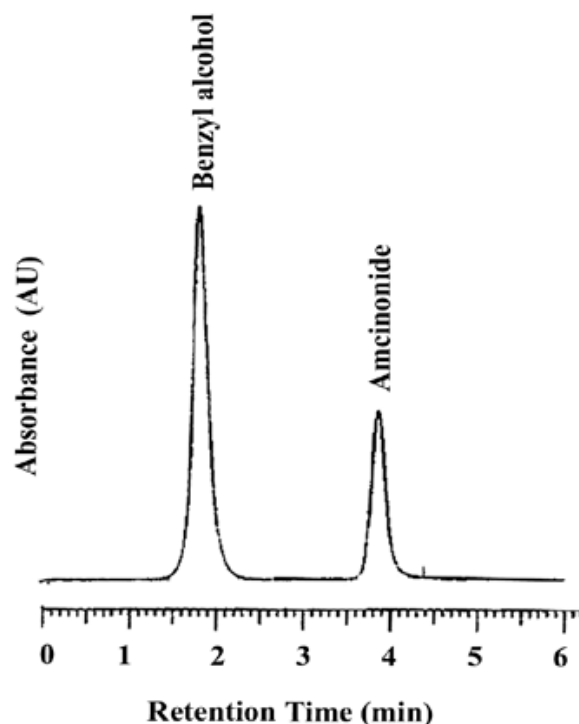

Fig. (1). Chromatogram of an authentic mixture containing 20 mg/ml amcinonide and 400 mg/ml benzyl alcohol. Chromatographic conditions: Instrumentation: a Shimadzu 6 A system (Schimadzu, Tokyo, Japan), comprising a LC-6A pump, a SCL-6A system controller, a SIL-6A auto-injector, a SPD-6A, an UV-detector and a C-R6A chromatopac; a Zorbax reversed-phase column (5µm, 250x4.6mm); temperature: ambient; mobile phase: water/ aceto-nitrile (70:30, v/v); linear gradient elution; flow rate: 1.5ml/min; detection: UV, λ = 254 nm.

Table 1.   **Regression Analysis of the Calibration Data**

Drug	Slope	Intercept	Correlation Coefficient (r^2)
Amcinonide	2245.8881	11616.5454	0.9997
Benzyl alcohol	2005.4363	10111.6363	0.9997

Average of three experiments.

The validity of the proposed method was tested by the assay of an authentic mixture containing known quantities of amcinonide and benzyl alcohol in pharmaceutical formulations at 80, 100 and 120% level of theoretical assay concentration. The results showed good accuracy (as revealed by the percentage recovery, see Table **2**), which clearly demonstrates that placebo of the formulation is not interfering with the assay method.

Precision was assessed by injecting the standard solution five times and by the five times assay of a homogeneous sample. The results obtained were found to be statistically valid (Tables **3** and **4**).

To determine the robustness of the method, the stability of the analytes was investigated. They were found to be stable in solution at ambient temperature for three days after their preparation with out losses as given in Table **5**.

Table 2. Simultaneous Determination of Amcinonide and Benzyl Alcohol

Amcinonide			Benzyl Alcohol		
Amount Added (mg/g)	Amount Found (mg/g)	Recovery (%)	Amount Added (mg/g)	Amount Found (mg/g)	Recovery (%)
0.8036	0.8001	99.57	16.0210	15.9713	99.69
0.8020	0.8010	99.87	16.1320	15.8012	97.95
1.0230	1.0281	100.37	20.2016	20.1021	99.50
1.0243	1.0050	98.81	20.4118	19.6101	96.07
1.0186	0.9901	97.72	20.3268	20.1248	99.01
1.0034	0.9710	96.77	20.4219	20.0175	98.02
1.2030	1.1926	99.14	24.3011	23.8614	98.82
1.2328	1.2528	101.64	24.1059	23.7823	98.87
Mean		99.24	Mean		98.49
SD		1.42	SD		1.08
% RSD		1.43	% RSD		1.10

Average of five experiments.

Table 3. Precision of Standard Solution Measurements

S. No.	Peak Area for Amicinonide (a.u.)	Peak Area for Benzyl Alcohol (a.u.)
1	37503	61973
2	37721	61924
3	37383	61093
4	38504	61842
5	38802	61936
Mean	37982	61753
SD	632	372
%RSD	1.7	0.6

Table 4. Precision of Test Sample Measurement

S. No.	Peak Area for Amicinonide (a.u)	Peak Area for Benzyl Alcohol (a.u)
1	99.20	100.35
2	97.31	101.00
3	98.46	102.00
4	99.38	103.10
5	98.31	103.21
Mean	98.53	101.93
SD	0.82	1.26
%RSD	0.84	1.24

Intermediate precision of the method was examined by two different operators. Each carried out three determinations of the same batch using the described HPLC method. The results are given in Table **6**. The developed HPLC method has been found to be time-saving as it provides the analysis of two compounds simultaneously with a high degree of precision and accuracy.

Table 5. Robustness of the Method

Time Period (h)	Amcinonide (%)	Benzyl Alcohol (%)
0	98.25	103.16
24	97.58	101.52
48	98.47	103.28

Table 6. Intermediate Precision of the Method

Operator	Determination	Amcinonide (%)	Benzyl Alcohol (%)
A	01	99.00	103.50
	02	97.20	101.31
	03	98.32	103.02
B	01	99.38	100.35
	02	98.48	102.02
	03	99.36	104.10
Mean		98.62	102.38
S.D		0.82	1.42
% RSD		0.84	1.38

The limits of detection (LOD) are for amcinonide and benzyl alcohol 2 µg/ml and 10 µg/ml whereas the limits of quantization (LOQ) lie within the range of 4-30 µg/ml and 80-600 µg/ml, respectively.

CONCLUSIONS

This paper describes a simple, rapid, economic, and accurate method of analysis using reversed-phase HPLC (RP-HPLC) for the simultaneous determination of amcinonide and benzyl alcohol in pharmaceutical preparations. A validation of this analytical method was carried out and showed that specificity, robustness and precision are guaranteed. In addition, linearity is ensured for contents between 4-30 and 80-600 µg/ml of amicinonide and benzyl alcohol, respectively, and the recovery was calculated to be in the range of 99 to 100%. The developed HPLC method is therefore appropriate for simultaneous and quantitative determinations of amicinonide-benzyl alcohol mixtures for quality assurance and R&D laboratories of pharmaceutical and cosmetic industries.

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