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Development and Validation of a Rapid HPLC Method for the Determination of Ketotifen in Pharmaceuticals

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ABSTRACT In the present study, a simple, sensitive, rapid, and stability-indicating high performance liquid chromatographic (HPLC) method with ultraviolet detection for the analysis of ketotifen was developed and validated. The method was applied to the determination of ketotifen in pharmaceutical formulations (tablets and syrups). The HPLC method utilized isocratic elution technique with a reversed phase C8 column, detection at 297 nm and a mixture of methanol, triethylamine phosphate buffer (pH 2.8; 0.04 M), and tetrahydrofuran (43: 55: 2, v/v/v) as mobile phase at a flow rate of 1.2 mL/min. Total analysis time was about 7 min with typical retention time of ketotifen of about 5 min. The method was validated for selectivity, linearity, accuracy, and precision following International Conference of Harmonization, 1996 (ICH) recommendations. Due to its simplicity and accuracy, the method can be used for routine quality control analysis.

KEYWORDS Ketotifen, Assay, High performance liquid chromatography (HPLC), Validation, Pharmaceuticals

INTRODUCTION

Ketotifen, 4,9-Dihydro–4-(1–methyl–4–piperidinylidene)-10*H*-benzo[4,5] cyclohepta-[1,2-*b*]thiophen–10–one, is an antiallergic drug with stabilizing action on mast cells, analogous to that of sodium cromoglycate, and anti-H1 effect (Sweetman, 2003). The anti-allergic action of ketotifen seems to be distinct from its antihistaminic properties (Sweetman, 2003). It is given orally as fumarate in the prophylactic management of asthma and is also used in the treatment of allergic conditions such as rhinitis and conjunctivitis (Sweetman, 2003; Alali et al., 2004). Several analytical methods carried out by high performance liquid chromatography (HPLC) to determine ketotifen were found in the literature, aimed at quantifying ketotifen in biological fluids (Alali et al., 2004; Chen et al., 2003), raw materials (Mikotic-Mihun et al., 1984; Moffat et al., 2004), and its related substances (European Pharmacopoeia, 2002). These methods utilized different ways of detection such as mass spectrometry

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(Alali et al., 2004; Chen et al., 2003) and UV detection (Mikotic-Mihun et al., 1984; Moffat et al., 2004; European Pharmacopoeia, 2002). Liquid chromatography with UV detection is often preferred in ordinary laboratories because of its wide availability and suitability. However, most methods described with UV detection involved complex chromatographic conditions such as gradient elution or long analysis times making them unsuitable for routine analysis. In addition, no High Performance Liquid Chromotography (HPLC) method was developed for the determination of ketotifen in pharmaceutical formulations. Several GC and GC/MS analytical methods were also found (Tzvetanov et al., 1999; Julien-Larose et al., 1983; Leis & Malle, 1991; Maurer & Pfleger, 1988; Grahnen et al., 1992; Sieradzki et al., 1989).

The target of this study was to develop and validate a new, simple, rapid, and stability-indicating HPLC method to determine ketotifen in raw materials and pharmaceutical formulations including tablets and syrups. Stability-indicating HPLC assays are of value in quality control and stability studies of drug substances and pharmaceutical formulations.

EXPERIMENTAL

Equipment

High performance liquid chromatography (HPLC) System was PerkinElmer Series 200 equipped with Series 200 LC pump, Online series 200 vacuum degasser, Series 200 autosampler, Series 200 UV/Vis detector, and Series 600 interface. The detector was set at a wavelength of 297 nm. Separation was carried out on Spheri-5, RP-8 column (PerkinElmer) with a particle size of 5 μm and 250 mm × 4.6 mm ID. Data acquisition was performed using TotalChrom Navigator 6.2.0.0, a computerized chromatography analysis software.

Materials and Reagents

Ketotifen fumarate was kindly supplied by Laboratori Alchemia (Milano, Italy). HPLC grade methanol was obtained from Riedel-de Haen AG (Seelze, Germany). High performance liquid chromatography (HPLC) grade tetrahydrofuran was obtained from Sds (Peypin, France). All other chemicals and reagents were of analytical grade.

Two commercial tablet formulations and two commercial syrup formulations were used. Formulations contained ketotifen fumarate equivalent to 1 mg ketotifen per tablet for tablet formulations and per 5 mL for syrup formulations.

Chromatographic Conditions

Isocratic elution technique was utilized with the C8 column maintained at room temperature. The mobile phase was a mixture of methanol, triethylamine phosphate buffer (pH 2.8; 0.04 M), and tetrahydrofuran (43: 55: 2, v/v/v). The buffer was prepared by adding 4.61 g ortho-phosphoric acid (85% w/w) and 3.57 g triethylamine to 900 mL water followed by mixing and fine adjusting of pH to 2.8 ± 0.1 with ortho-phosphoric acid. Finally, it was diluted to 1000 mL with water. The mobile phase was filtered through a 0.45 μm membrane filter and degassed prior to use. The flow rate was 1.2 mL/min. These conditions were selected among others investigated as leading to optimal resolution between ketotifen and its degradation products or excipients in formulations, optimal peak shape, as well as for convenience regarding total time of analysis.

All samples were filtered through a 0.45 μm membrane filter. The injection volume was 50 μL and detection was performed at a wavelength of 297 nm.

Stock and Standard Solutions

Accurately weighed amount of ketotifen fumarate was dissolved in a solvent mixture of methanol and triethylamine phosphate buffer (pH 2.8; 0.04 M) (40:60; v/v) to prepare a stock solution in a concentration of 200 μg/mL which is equivalent to ketotifen base concentration of 145.43 μg/mL. Standard solutions were prepared by dilution of the stock solution with the same solvent mixture to give solutions containing ketotifen fumarate equivalent to ketotifen base in the concentration range of 0.15–145.43 μg/mL.

Sample Solution

Solvent mixture of methanol and triethylamine phosphate buffer (pH 2.8; 0.04 M) (40:60; v/v) was used to prepare and dilute all sample solutions. This mixture in these proportions was selected among others investigated as leading to optimal extraction of the

drug from formulations. For each of the tablet formulations, ten tablets were weighed to obtain the average tablet weight and crushed to fine powder. Powder samples, equivalent to 1 mg ketotifen base, were placed in a 50-mL volumetric flask. Aliquot, 40 mL solvent mixture, were added and sonicated for 5 min. The mixture was then diluted to 50 mL with the solvent mixture and thoroughly mixed to produce a final ketotifen base concentration of 20 μg/mL. For syrup formulations, samples equivalent to 1 mg ketotifen base were diluted to 50 mL with the solvent mixture and thoroughly mixed to produce a final ketotifen base concentration of 20 μg/mL. Prior to injection, all samples were passed through a 0.45 μm membrane filter.

RESULTS AND DISCUSSION

System Suitability

A system suitability test was defined based on the results obtained in several representative chromatograms. The values of the number of theoretical plates were higher than the accepted value of 2000. The tailing factor was less than 2.2. The relative standard deviation (RSD) for five replicate injections of standard solutions of ketotifen fumarate equivalent to ketotifen base concentration of 7.27, 36.36, 72.71 μg/mL was less than 1%.

Selectivity

Selectivity of the described method was determined by analyzing forcedly degraded samples. Forced degradation studies were performed to provide evidence of the stability-indicating property of the proposed method. Intentional degradation was achieved by exposing drug solution to stress conditions of acid (ketotifen fumarate equivalent to ketotifen base concentration of 290.86 μg/mL in 1 M HCl at 70°C for 3 hours) and base (ketotifen fumarate equivalent to ketotifen base concentration of 290.86 μg/mL in 1 M NaOH at 70°C for 3 hours) in order to test the ability of the proposed method to separate the active components from degradation products. After exposure to stress conditions, solutions were neutralized and then diluted using solvent mixture of methanol and triethylamine phosphate buffer (pH 2.8; 0.04 M) (40:60; v/v). Under basic conditions, free ketotifen base, which is insoluble in water (Mikotic-Mihun et al., 1984), was liberated and precipitated. This precipitate was found to completely dissolve after neutralization and dilution using the solvent mixture. Under forced degradation study in acidic conditions, the presence of an unknown degraded product, eluted at approximately 2.53 min, was noticed. However, the decomposition was not significant as the percentage of ketotifen recovered was more than 95%. Forced degradation study in basic conditions resulted in significant decomposition where the percentage of ketotifen recovered was 3.6%. This suggests poor stability of the drug under basic conditions. Chromatograms for acid and base degradation are individually shown in Fig. 1B-C. These chromatograms show that degradation products could be well resolved from the parent component and that the proposed method displays satisfactory selectivity to ketotifen and its degradation products.

The selectivity of the developed method for the determination of ketotifen in pharmaceutical preparations was also investigated. Typical chromatograms

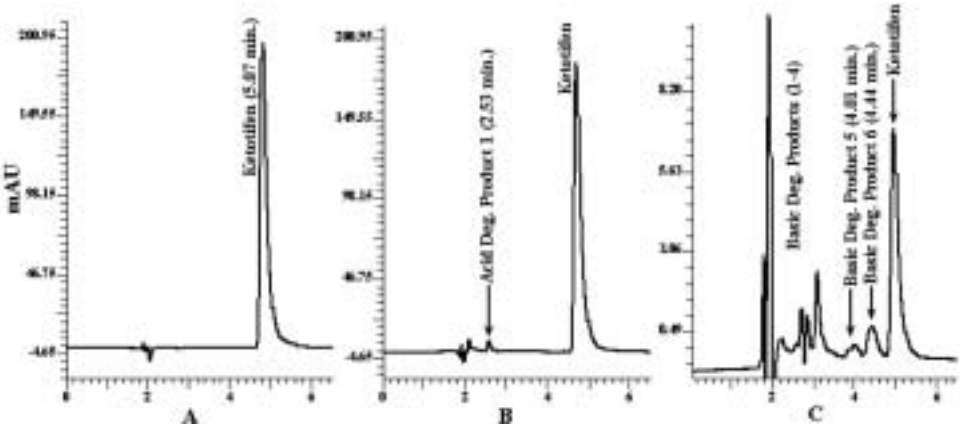

FIGURE 1 HPLC Chromatograms of Ketotifen (36.36 μg/mL) (A); Acid Degradation (B) Base Degradation (C).

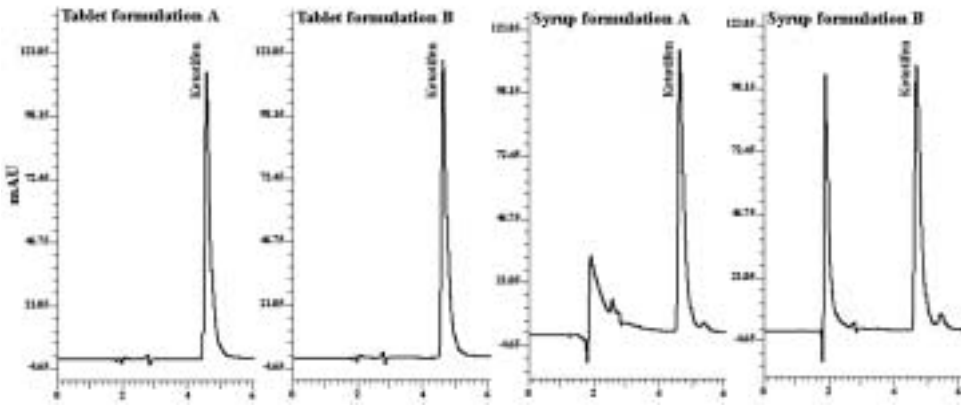

FIGURE 2 **HPLC Chromatograms of Ketotifen in Pharmaceutical Preparations.**

obtained from pharmaceutical preparations are shown in Fig. 2. These chromatograms show that excipients in pharmaceutical preparations did not interfere with the active component. The ability of the method to separate the drug from its degradation products and excipients in pharmaceutical preparations indicated good selectivity of the developed method.

Linearity

Five solutions containing ketotifen fumarate equivalent to ketotifen base at concentrations ranging from 0.73–145.43 μg/mL were analyzed. The peak area versus concentration curve proved to be linear. The regression line equation calculated by least-squares method was $A = 139752.49\ C - 119388.23$ with a coefficient of correlation $r = 0.99995$ and slope *RSD* (five determinations) of 0.28%. The results show that within the concentration range tested there was an excellent correlation existing between peak area and concentration of the drug.

Precision and Accuracy

Precision was considered at two levels according to ICH recommendations (International Conference of Harmonization, 1996): repeatability and intermediate precision. In order to verify accuracy and repeatability, replicate injections of standard solutions at low (7.27 μg/mL), medium (36.36 μg/mL), and high (72.71 μg/mL) concentration levels were conducted. The accuracy was calculated as the percentage of the analyte recovered by the assay (Table 1). The results indicated that the mean recovery was 99.43% with overall RSD of 1.06%. The experimental *t* of the recovery percentage, whose value was 1.611, was far below the value of 2.306 established in the tabulated *t* (α:0.05; 8 d.f.) indicating that the experimental average was not significantly different from the true value. These results indicated the sufficient accuracy and repeatability of the proposed analytical method.

TABLE 1 **Recovery Analysis**

Quantity added (μg/mL)	Average Quantity found (n = 3) (μg/mL)	Average Recovered (n = 3) (%)	RSD (%)
7.27	7.21	99.39	0.55
36.36	35.75	98.32	0.49
72.71	73.14	100.58	0.31
Means		99.43	1.06

Intermediate precision was determined by carrying out two accuracy assays on the same lot of each of the commercial formulations on different days. For each formulation, the samples were taken at three different levels and three replicate determinations were made at each level. Values of the assay results, expressed as percentage of the label claim, were used to evaluate the precision of the method (Table 2). Recovery data obtained from the study of tablet formulation *A* ranged from 104.14% to 111.34% with a mean value of 108.69% ($n = 9$) and overall RSD of 2.37%. Tablet formulation *B* data ranged from 104.20% to 106.04% with a mean value of 105.48% ($n = 9$) and overall RSD of 0.64%. Syrup formulation *A* data ranged from 97.78% to 101.57% with a mean value 99.50% ($n = 9$) and overall RSD of 1.37%. Syrup formulation *B* data ranged from 95.15% to 97.31% with a mean value

TABLE 2 Intermediate Precision of the Assay Method

Formulation	% w/w[a]	Average recovered (%)	RSD (%)
Tablet formulation A	75	110.96	0.50
	100	108.39	0.17
	125	105.19	0.91
Means (n = 9)		108.69	2.37
Tablet formulation B	50	105.51	0.74
	100	105.96	0.08
	150	104.91	0.59
Means (n = 9)		105.48	0.64
Syrup formulation A	75	98.78	0.89
	100	100.78	0.70
	150	98.30	0.75
Means (n = 9)		99.50	1.37
Syrup formulation B	75	96.18	1.51
	100	96.92	0.65
	150	96.37	0.08
Means (n = 9)		96.55	0.81

[a]100% is corresponding to final sample solution of ketotifen base concentration of 20 μg/mL.

96.55% ($n = 9$) and overall RSD of 0.81%. The above results indicated the sufficient accuracy and precision of the proposed analytical method.

Limit of Quantitation

The limit of quantitation was determined as described according to ICH guidelines (International Conference of Harmonization, 1996) by the experimental analysis of different samples with known concentrations of analyte. The minimum concentration at which the analyte could be quantified with acceptable accuracy and precision was found to be 0.60 μg/mL which corresponds to 3% of the sample solution concentration.

Solution Stability

Standard and sample solutions injected after 3 days of storage at refrigerator (2–8°C) failed to show any appreciable change.

CONCLUSION

A simple, rapid, accurate, precise, selective, and stability-indicating new analytical method has been developed and validated for the routine determination of ketotifen in raw materials and pharmaceutical formulations. Selectivity was demonstrated showing that ketotifen peak was free of interference from degradation products. Thus, the proposed method can be used in routine stability assay. Ketotifen peak was also free from interference from excipients of pharmaceutical formulations indicating selectivity of the method for the determination of ketotifen in pharmaceutical formulations. The linearity of ketotifen peak area responses was demonstrated in a concentration of ketotifen base in the range of 0.73–145.43 μg/mL by a correlation coefficient of 0.99995. According to recovery studies performed at concentrations ranging from 50–150% of the analytical concentration, the extraction of the active component was shown to be quantitative and the analytical method proved to be accurate and precise.

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