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Validated Stability-Indicating RP-LC Method for the Determination of Pemirolast Potassium Investigation of Kinetic Behavior

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Abstract

The stability of the anti-asthmatic drug pemirolast potassium was investigated under different stress conditions, including hydrolysis (acid and alkali), heat, light and oxidation as recommended by the ICH guidelines. Degradation process was found to take place only under alkaline and oxidative conditions. A stability-indicating RP-LC method was developed and validated for the determination of pemirolast potassium in the presence of its degradation products and process-related impurity. The chromatographic analysis was achieved on Eclipse®, XDB-C_{18} (150mm x 4.6 mm, 5µm) column, under isocratic elution by a mixture of (water: methanol: glacial acetic acid, pH 3.5) 50:50:0.3 (*v/v/v*), as a mobile phase, delivered at 1.0 ml/min at 258 nm. Method validation demonstrated to be selective, accurate and precise with good linearity over the concentration range of (1-10 µg/ml) with limits of detection and quantification of 21 and 69 ng/ml, respectively. Robustness against small modifications of pH and the percentage of the aqueous mobile phase was ascertained. The developed method was successfully applied for the analysis of pemirolast potassium in commercial eye drops and tablets; therefore it's highly suitable for routine analysis in QC labs. Moreover this method was utilized to investigate the kinetic of the alkaline degradation of pemirolast potassium, determine the order of the degradation rate constant, calculate the rate constant, half-life time and estimate the drug shelf-life (expiry date), and the activation energy of the degradation process.

Keywords: Pemirolast potassium; Stability-indicating RP-LC method; Method Validation; Degradations; Process-related impurity; Kinetic.

1. Introduction

Pemirolast potassium (PP) is a mast cell stabilizer and a leukotriene inhibitor. Chemically, pemirolast potassium is (9-methyl-3-(1 H-tetrazol-5-yl)-4H-pyrido [1, 2-α] pyrimidin-4-one potassium) Fig.1. It has been used in the treatment of chronic asthma and in the prophylaxis of allergic rhinitis and conjunctivitis [1]. Pemirolast potassium is not official in any pharmacopeia; literature survey revealed that some HPLC methods were reported for the determination of Pemirolast potassium in human plasma and in pharmaceutical products [2-9]. To the best of our knowledge; in the literature there is no reported stability-indicating method for the determination of pemirolast potassium in the presence of its degradation products and process-related impurity, moreover, there is no reported method investigate the kinetics of the degradation or explain the degradation behavior of the drug, so, the primary objective of this study was to develop an accurate, simple, specific, reproducible, systematic and reliable stability-indicating HPLC method free of placebo interferences to separate and quantify pemirolast potassium in the presence of its degradation products and process-related impurity in a short run time, wherefore; it can be applied during degradation product monitoring studies. The present manuscript described the (i) degradation behavior of pemirolast potassium under alkaline hydrolysis and oxidation stress condition, (ii) optimization of RP-LC conditions to separate the drug, its forced alkaline degradation products and process-related impurity, (iii) method validation and (iv)Kinetic investigation of the alkaline degradation.

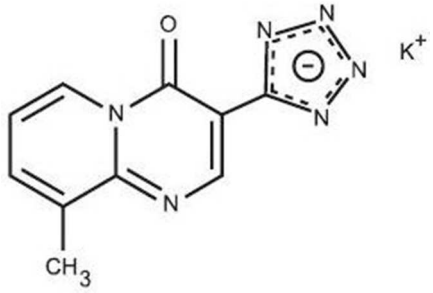

Fig.1 Chemical structure of Pemirolast Potassium

Results and discussion

Optimization of the chromatographic conditions

The goal of this study is to develop a systematic, reproducible, robust and reliable stability-indicating RP-LC method to separate and quantify pemirolast potassium in the presence of its degradation products and process-related impurity, so, an efficient and comprehensive experimental design based on systematic scouting of the three key HPLC method components including column, mobile phase, and pH conditions was constructed (Tab. 1). An experimental design of a set of 2 column, 4 mobile phases, and 3 pH values was developed. These chromatographic conditions enable the creation of a database that describes the relationship of the compound retention and possible RP-LC conditions. The method conditions were evaluated for peaks symmetry, peaks fronting, peaks tailing and the resolution. The final method conditions were selected and the evaluation of the test robustness was carried out with appreciate system suitability criteria to improve the overall understanding of the method performance under various conditions.

Tab. 1 Scouting of three parameters of HPLC

Parameters	Description of parameters
Column	C8 C18
Mobile phase	ACN:Water ACN:Water (pH 3.5) Methanol:Water Methanol:Water (pH 3.5)
pH (water), adjusted with glacial acetic acid	3.5 4.5 5.5

Method development for C8

All the trials are shown in tab. 2.

Tab. 2 Observations and remarks of method development for C8

Trial No.	Conditions	Observation	Remarks
1	ACN:Water (35:65%, v/v)	Peak was found to be asymmetrical.	Not Satisfactory
2	ACN:Water (35:65%, v/v pH 3.5)	Peak was found to be asymmetrical.	Not Satisfactory
3	Methanol:Water (35:65%, v/v)	Peak was found to be asymmetrical.	Not Satisfactory
4	Methanol:Water (35:65%, v/v pH 3.5)	Sharp peaks were obtained but tailing factor was more than 1.5 and no complete resolution was observed	Not Satisfactory

Method development for C18

All the trials are shown in tab. 3.

Tab. 3 Observations and remarks of method development for C18

Trial No.	Conditions	Observation	Remarks
1	ACN:Water (35:65%, v/v)	Peak was found to be asymmetrical.	Not Satisfactory
2	ACN:Water (35:65%, v/v pH 3.5)	Peak was found to be little asymmetrical and tailing was obtained.	Not Satisfactory
3	Methanol:Water (35:65%, v/v)	Peak was found to be asymmetrical.	Not Satisfactory
4	Methanol:Water (35:65%, v/v pH 3.5)	Good symmetrical sharp peaks with accepted tailing factors and resolutions.	Satisfactory

Method development for C18 using different pH conditions

All the trials are shown in tab. 4.

Tab. 4 Observations and remarks of method development for C18 in various pH conditions

Trial No.	Conditions	Observation	Remarks
1	Methanol:Water (35:65%, v/v pH 4.5)	Peak was found to be asymmetrical.	Not Satisfactory
2	Methanol:Water (35:65%, v/v pH 5.5)	Peak was found to be very asymmetrical.	Not Satisfactory
3	Methanol:Water (35:65%, v/v pH 3.5)	Good symmetrical sharp peaks with accepted tailing factors and resolutions but with long run time (40 min.).	Not Satisfactory
4	Methanol:Water (50:50%, v/v pH 3.5)	Good symmetrical sharp peaks with accepted tailing factors and resolutions with short run time (20 min.).	Satisfactory

Final Method Conditions

Column: Eclipse®, XDB-C_{18} column (150mm x 4.6 mm, 5µm)
Mobile Phase: Methanol: water: glacial acetic acid, pH 3.5) (50:50:0.3, *v/v/v*)
Flow Rate: 1 ml/min.
Oven Temperature: 25˚ C
Detection: DAD at 258 nm
Injection Volume: 10 µl. Typical chromatograms are shown in Fig 2-7.

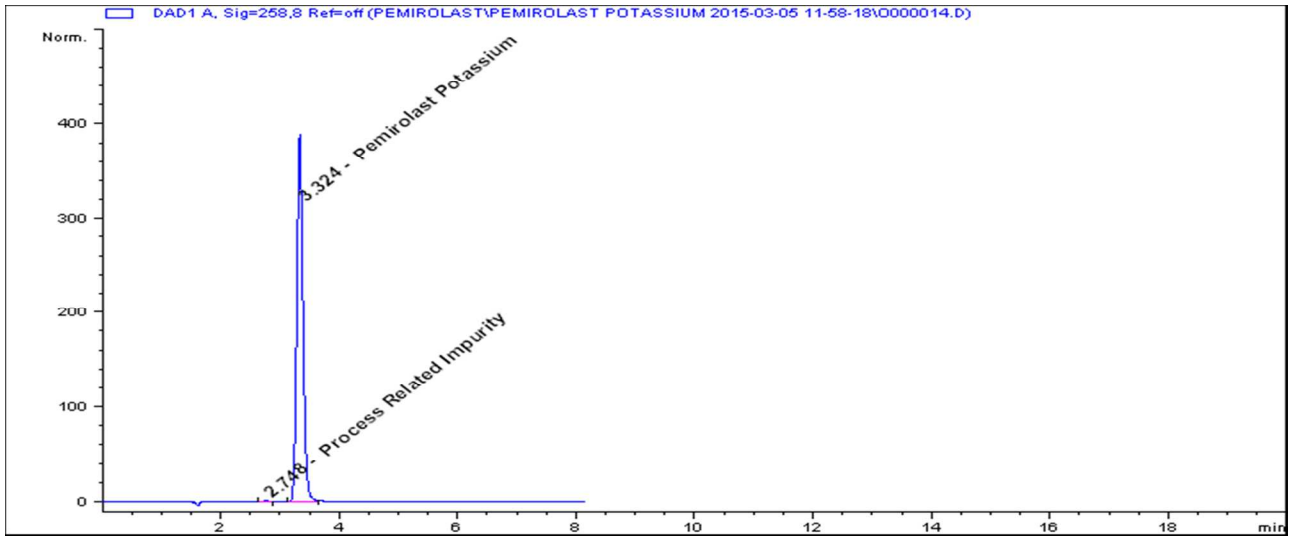

Fig.2 Chromatogram of pemirolast potassium standard

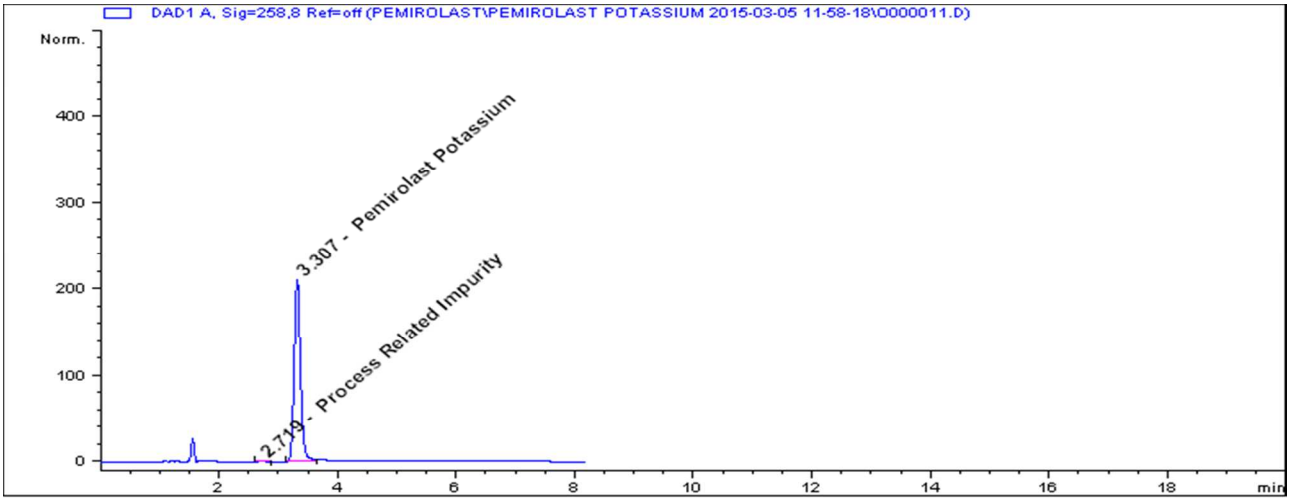

Fig.3 Chromatogram of pemirolast potassium after reflux with 1 M HCl for 2 hours

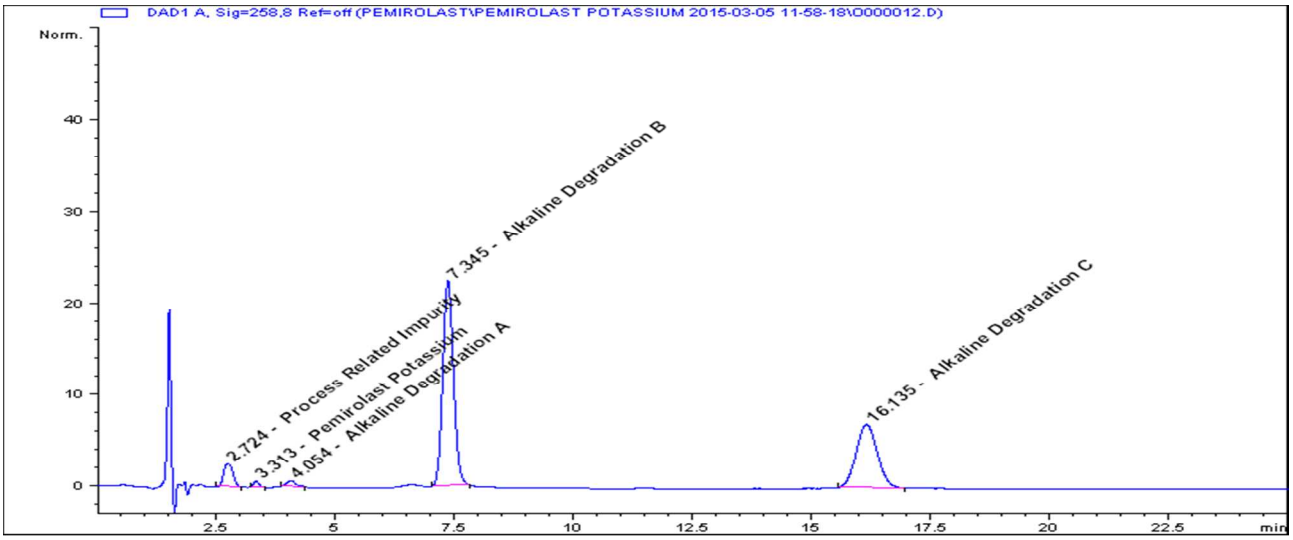

Fig.4 Chromatogram of pemirolast potassium after reflux with 0.1 M NaOH for 2 hours

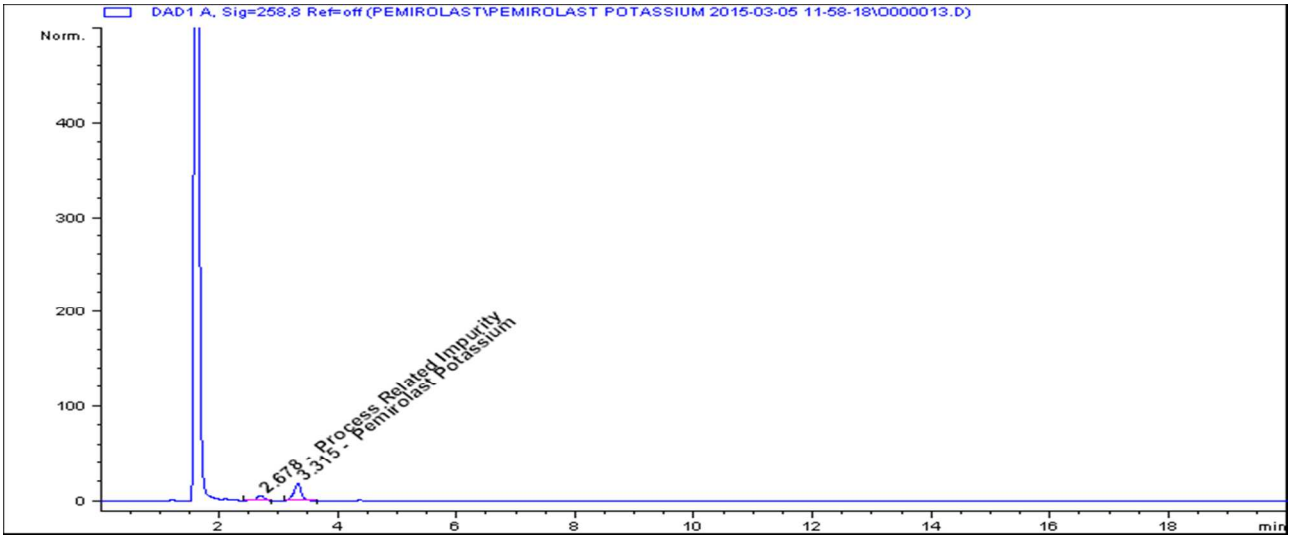

Fig.5 Chromatogram of pemirolast potassium after reflux with 5% H_2O_2 for 2 hours

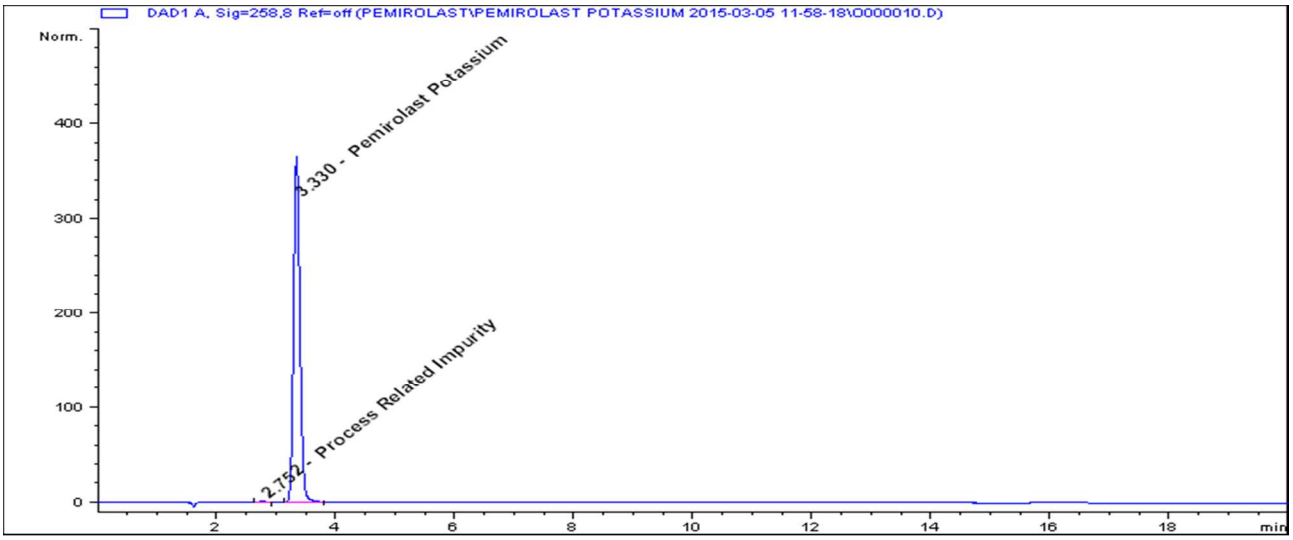

Fig.6 Chromatogram of pemirolast potassium after heating at 105 °C for 5 hours

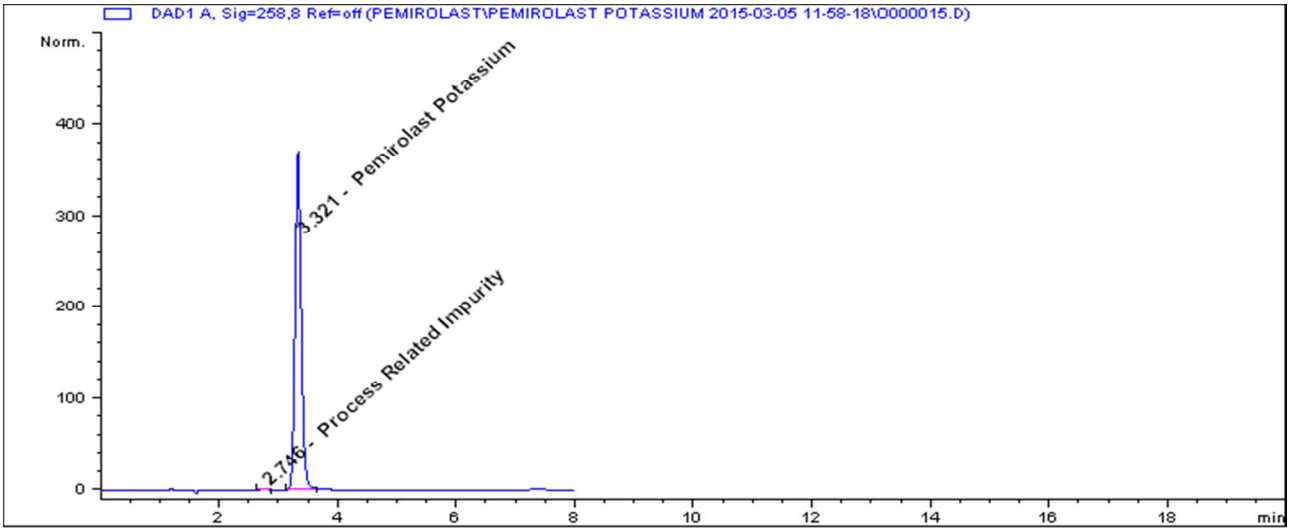

Fig.7 Chromatogram of pemirolast potassium after exposing to light for 5 hours

Method validation

After satisfactory development of the method, it was subjected to method validation as per ICH guidelines [10]. The method was validated to demonstrate that it is suitable for its intended purpose by the standard procedure to evaluate adequate validation characteristics (system suitability, specificity, accuracy, precision, linearity, robustness, ruggedness, LOD and LOQ and stability-indicating capability).

System suitability

The system suitability parameters (Tab. 5) were evaluated by making the injection of the assay standard. The system was deemed to be suitable as the tailing factors for all the peaks were between 0.8 and 1.5 and the resolution between PP, the process-related impurity and the alkaline degradation products was > 2.5.

Tab.5 The results for the system suitability of the assay standard

Component	Tailing factor	USP plate count	Resolution	Selectivity
Process-related impurity	0.85	2077	-	-
Pemirolast potassium	0.81	4624	2.07	1.22
Alkaline degradation A	0.85	2043	2.67	1.22
Alkaline degradation B	0.87	5189	8.57	1.81
Alkaline degradation C	0.93	6260	14.34	2.20

Specificity

In order to determine whether the developed analytical method was stability-indicating, pemirolast potassium was exposed to various stress conditions (acid hydrolysis, base hydrolysis, oxidation, thermal stress and sunlight) to conduct forced degradation studies as per ICH guidelines [10]. PP is very soluble in water. The acidic, basic, and oxidative stress condition studies were carried out by refluxing pemirolast potassium for 2 hours with 1 M HCl, 0.1 M NaOH, and 5 % H_2O_2 respectively. The thermal stress was carried out by heating pemirolast potassium at 105 °C for 5 hours and the photodegradation was performed by exposing pemirolast potassium to sunlight for 5 hours. It is interesting to note that all the peaks due to degradation products were well-resolved with good tailing factors. The chromatograms of the stressed samples were evaluated for peak purity using Agilent software Chemstation 32. For all forced degradation samples, the purity factor of pemirolast potassium exceeded the calculated threshold limit. This confirms the stability-indicating power of the developed method. The results of the forced degradation study are shown in Tab. 6.

Tab.6 The results table for the spasticity of pemirolast potassium

Sample No.	Stress Conditions	Purity Factor	Calculated Threshold Limit
1	**Sample without effect**	**999.079**	**848.050**
2	**Acid stress**	**999.794**	**420.010**
3	**Base stress**	**908.311**	**7.390**
4	**Oxidation stress**	**935.259**	**32.430**
5	**Sunlight stress**	**999.222**	**795.500**
6	**Thermal stress**	**999.318**	**786.990**

Precision

The precision of the test method was evaluated by analyzing three different concentrations of PP; three replicates of each concentration were analyzed within a day to determine the intra-day precision and over three days to determine the inter-day precision. The RSD was found to be below 2.0% which indicates the precision of the method for the quantification of pemirolast potassium. The results are shown in Tab.7.

Tab.7 The results table of the precision of the test method

Conc (μg/ml)	Recovery ± RSD%	
	Intra-day	**Inter-day**
1	99.21 ± 1.031%	99.42 ± 0.529%
4	98.81± 1.197%	98.34 ± 1.784%
8	98.91 ± 0.801%	99.48 ± 0.993%

Limits of detection and quantification

The limit of detection and the limit of quantification were established based on residual standard deviation of the response and the slope. The LOD and LOQ were found to be 21 and 69 ng/ml. respectively.

Linearity

Good linearity was established in the concentration range (1-10 µg/ml) of PP. The data was subjected to statistical analysis using linear regression model; the linear regression equation was Y= 59.4819x + 2.1770. The linear graph was plotted for the concentration (µg/ml) versus detector response (area). The correlation coefficient was found to be 0.9995. Linearity plot is shown in Fig. 8.

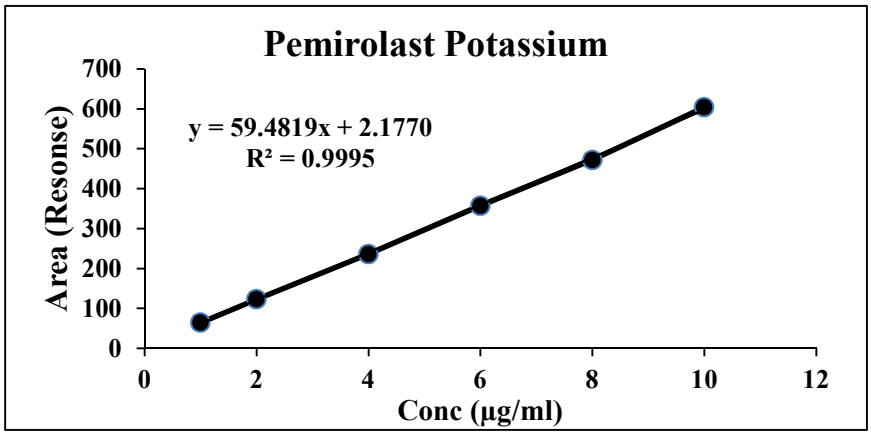

Fig.8 Linearity of detector response (area) of pemirolast potassium

Accuracy

Accuracy study for PP was conducted using five different concentrations within the linearity range (3, 5, 7, 9, 10 µg/ml). Three replicates of each concentration were analyzed and % recovery was found to be between 98.0 % and 102.0%. The results are tabulated in Tab. 8.

Tab.8 The results table of the accuracy of the test method

Conc (μg/ml)	Recovery	± RSD%
3	98.517%	0.975%
5	98.839%	1.277%
7	99.243%	1.116%
9	98.687%	0.454%
10	98.291%	0.597%

Robustness

The robustness was investigated by varying the conditions with respect to the flow rate, column temperature and pH of the mobile phase. The study was conducted at three different flow rates (0.95 ml/min, 1.00 ml/min and 1.05 ml/min), at three different column temperatures (20°C, 25°C and 30°C) and at three different pH (3.4, 3.5 and 3.6) to study the effect of these changes on the different chromatographic parameters. Negligible difference was found in system suitability parameters for pemirolast potassium such as USP plate count, resolution between pemirolast potassium and the process-related impurity and the tailing factor (Tab. 9), so the method was found to be robust.

Tab. 9 System suitability results from robustness

Parameters	Flow rate (ml/min)			Column temperature (°C)			pH		
	0.95	1.00	1.05	20	25	30	3.4	3.5	3.6
USP plate count	5024	5058	5064	5012	5058	5074	5028	5058	5017
Resolution	3.18	3.21	3.29	3.24	3.25	3.33	3.23	3.25	3.21
Tailing factor	0.845	0.851	0.864	0.851	0.851	0.86	0.84	0.851	0.847

Kinetic of Pemirolast potassium alkaline degradation

Pemirolast potassium samples were collected at different time intervals under various stress conditions. Pemirolast potassium was stable under acidic, thermal and sunlight stress conditions even after 2 hours. However on alkaline and oxidative hydrolysis, pemirolast potassium degraded rapidly within 2 hours and 45 min. respectively. Hence, the kinetic of the basic degradation of Pemirolast potassium was carried out at [50° C- 60° C-70° C], the Arrhenius plot for the 1/T (K^{-1}) versus; K_{obs} ($hour^{-10}$) was drawn, (Fig. 9) and the half-life (hour), degradation rate constant K_{obs} ($hour^{-10}$), and activation energy; E_a (kJ/mol) of the degradation process were calculated. Besides, typical pharmaceutical parameter that describe the expiry date estimation of the drug (shelf life t90) was also calculated (Tab. 10). The alkaline degradation reaction of PP was found to follow a pseudo first-order kinetic.

Tab.10 Results of the Kinetic of the basic degradation of pemirolast potassium

Temperature (K)	Degradation rate constant K_{obs} ($hour^{-10}$)	Rate constant half-life (hour)	Shelf-life (t90) (hour)
323	0.001	0.525	0.350
333	0.002	0.825	0.125
343	0.004	2.310	0.080
activation energy E_a (kJ/mol)		195.540	

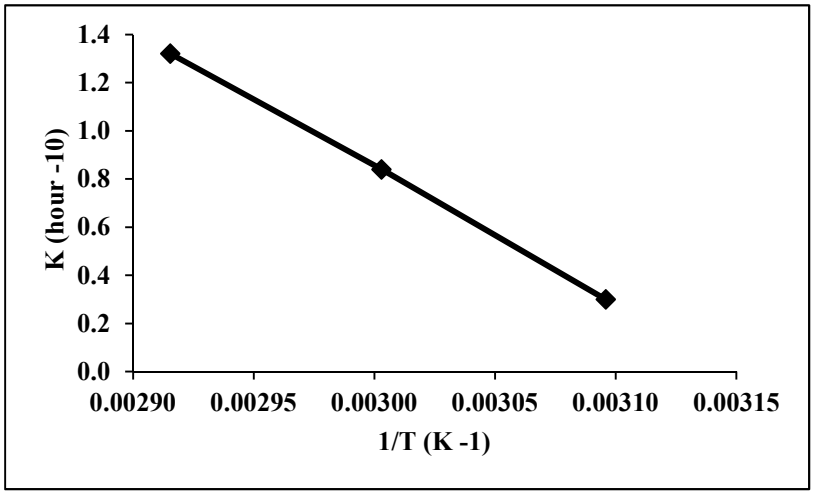

Fig. 9 Arrhenius plot for the alkaline degradation of pemirolast potassium

Experimental

Materials

Pemirolast potassium was supplied by Chemswiss AG, Switzerland.

Alegysal® 10 mg Tablets, Mitsubishi Tanab Pharma Corporation, Japan

Mirolast® 0.1% eye drops, Sigma Tec Co., for pharmaceutical industries, labeled to contain 1 mg/ml Pemirolast potassium, was purchased from the local market in Egypt

Methanol, HPLC grade (Labscan, Ltd, Dublin, Ireland)

Acetonitrile, HPLC grade (Labscan, Ltd, Dublin, Ireland)

Glacial acetic acid, HPLC grade (Sigma Aldrich, Germany)

Glass-distilled water

Apparatus

An Agilent technologies LC system 1200 series consists of quaternary pump equipped with DAD detector, auto injector and degasser. A thermostatic reversed phase Eclipse®, XDB-C_{18} column compartment (150mm x 4.6mm, i.d. 5µm) was used for separating all the compounds. The chromatographic data were recorded using Dell computer system with LC solution data acquiring software (Chemstation 32, USA)

Chromatographic conditions

All the samples were analyzed by HPLC on Eclipse®, XDB-C_{18} column using (methanol: water: glacial acetic acid, pH 3.5) (50:50:0.3, *v/v/v*) as a mobile phase, in isocratic elution at a flow rate 1 ml/min. at ambient column temperature. The detection was carried out by DAD at 258 nm. The mobile phase was filtered through a 0.45 µm nylon membrane filter.

Preparation of Standard

PP Stock Preparation

0.1 mg/ml of PP solution was prepared in water.

Standard Preparation (For assay)

1.0 ml of PP stock solution was transferred into a 10-ml volumetric flask and diluted with water and mixed well.

Preparation of degradation products

Forced degradation of PP was carried out according to ICH guidelines. About 10 mg/ml of PP was subjected to acidic, basic, heat, light and oxidation conditions by refluxing with 100 ml of 1M HCl, 0.1 M NaOH, water and 5%H_2O_2 for 2 hours. The thermal stress was carried out by heating pemirolast potassium at 105 °C for 5 hours and the photodegradation was performed by exposing PP to sunlight for 5 hours. The degradation products of acid and base hydrolysis were neutralized with NaOH and HCl, respectively. While oxidative degradation was evaporated on a boiling water bath. The samples were further diluted ten times with distilled water and filtered through 0.45 µm filter before HPLC analysis.

Sample Preparation

About 1.0 ml of Mirolast® eye drops was transferred into 100-ml volumetric flask and diluted with water and mixed well.

Twenty tablets of Alegysal® 10 mg tablets were weighed and grinded, an accurate weight equivalent to the average weight of one tablet was transferred into 100-ml volumetric flask, 60 ml of water were added & sonicated for 30 minutes. The tablet's extract was cooled then the volume was completed with the same solvent, homogenized and filtered through Millipore® filter (0.45 µm). 1 ml was accurately transferred into a 10-ml volumetric flask and the volume was completed with the same solvent.

Conclusion

A systematic, robust, validated and reliable isocratic stability-indicating HPLC method was developed for the determination of pemirolast potassium without any interference of blank, placebo, the process-related impurity and the degradation products, thereby affirming the stability-indicating nature of the method. Furthermore, the paper was utilized to investigate the kinetics of the alkaline degradation of the drug, determine the order of the degradation rate constant, calculate the rate constant, calculate the half-life time and estimate the drug shelf-life (expiry date) and activation energy of the degradation process. The proposed method was started with clear goals and the experimental design describes the scouting of the key HPLC method components. Their relationships are studied and the preliminary optimized conditions are obtained. All the validation parameters were found within accepted criteria. This developed method can be applied successfully to the quality control of the commercials and other routine analysis.

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