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Original Research Article

Fast and Accurate Quantitative Determination of Prednisolone and Chlorpheniramine in Active Pharmaceutical Ingredients and Parenteral Dosage Forms

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ABSTRACT

The approaches for quantitative drug analysis utilizing HPLC is hectic and time-consuming. The present research was conducted to develop an efficient and facile method for the simultaneous quantitative analysis of chlorpheniramine (CPM) and prednisolone (Pred) with reasonable accuracy and precision both in active pharmaceutical ingredient (API) and its parenteral dosage forms. C18 (5µm x 150 mm 4.6 mm) HPLC column with 50:50 v/v acetonitrile: buffer (0.008M sodium dihydrogen phosphate [NaH_2PO_4]) as mobile phase was used. The run time for the analysis was 10 min, while the retention times of CPM and Pred were 1 and 4 min, respectively. The developed method showed linearity, intermediate precision, and accuracy within the defined set limits according to international standards. Verification of this method was done on the parenteral dosage forms of both drugs. Linearity with R^2 values of 0.991607 for CPM and 0.990061 for Pred was obtained. Limit of detection and limit of quantification for Pred were calculated to be 0.033 and 0.11 mg/mL, while for CPM, 0.08 and 0.27 mg/mL were obtained. Accuracy parameter outcomes for Pred were within the range of 98.16576 to 98.46571% and the values for CPM were 98.10 to 99.57%. The validation results of the developed method are accurate and within system suitability parameters provided by International Council for Harmonization ICH and pharmacopeias.

Keywords: Chlorpheniramine, HPLC, Pharmaceutical chemistry, Prednisolone.

Introduction

Pharmaceutical medicine is a medical discipline concerned with the discovery, evaluation, registration, monitoring and clinical aspects of pharmaceutical development. Most of the active components of medicine are derived from natural sources like plants, herbs, or animals. Some of the active components of medicines are made synthetically from different sources. A class of drugs can be further classified into sub-classes based on their chemical composition, duration of action, or the receptors in the body. Antihistamines are the chemical agents that block the action of histamine by blocking histamine receptors.[1] They are antagonists to histamine receptors. Histamine receptors are G-protein coupled receptors in which the primary binding ligand is histamine.

At least four types of histamine receptors are present in the human body. They are named H_1, H_2, H_3, and H_4 receptors. H_1 are one of the most important receptors for modulating the internal clock and are the main target for many clinical drugs. Excess activation of these receptors triggers the symptoms of hay fever and other seasonal allergies.[2] H_2 are found on parietal cells located in the stomach lining and are mainly responsible for regulating the levels of gastric acid. Histamine can also inhibit antibody and cytokine production by reacting with these receptors.[3] H_3 are present throughout the nervous system, though most notably in the central nervous system.[4,5] The more of these receptors are triggered by histamine, the less histamine is produced in the body.[6,7]

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H_4 receptors regulate the levels of white blood cell release from the bone marrow.[8] They are located in the thymus, small intestine, and express similar expression configuration in the human oral epithelium spleen,[9] the colon, bone marrow, and basophils.[10-12] Antihistamines are great at soothing side effects of a hypersensitive response, for example, sneezing, running nose, edema, swelling, rashes, itching of eyes and irritation, etc.[13] They are more potent but cause more sedation. These drugs are very effective in the treatment of severe allergies.[14,15]

Chlorpheniramine or chlorphenamine (shortly CP or CPM) is a drug that belongs to a primitive class of antihistamine.[16] It is widely used in the treatment of skin allergies,[17] and can be combined with other drugs for the treatment of medical ailments like cough and cold. The drug is also commonly described as possessing weak anticholinergic activity by acting as an antagonist of the muscarinic acetylcholine receptors. In addition to acting as an inverse agonist at the H_1 receptor,[18] chlorphenamine has been found to act as a serotonin reuptake inhibitor (SRI) (K_d = 15.2 nM for the transportation of serotonin.[19] It has only a weak affinity for the norepinephrine and dopamine transporters.[20] Absorption is through the gut after first-pass metabolism by the liver. The drug is bound to plasma protein up to 72%. Chlorpheniramine is mainly metabolized and excreted in the urine as mono and di desmethyl chlorpheniramine and a small amount of drug is excreted unchanged. Prednisolone (Pred) is a glucocorticoid, both naturally occurring and synthetic that is absorbed from the gastrointestinal tract and is widely used for the treatment of various medical conditions like inflammation, autoimmune disorders,[21] adrenocortical insufficiency, uveitis, skin conditions, and dermatitis. The drug is a biologically active steroid that acts mainly as a glucocorticoid.[22,23] It alters the biochemical reactions of lipids, carbohydrates, and proteins. Also, it alters and ceases the formations of DNA. The mechanism of action in the control of inflammation is by blocking the production of prostaglandins and leukotrienes by inhibition of arachidonic acid. The activity of prednisolone in immune suppression is by binding to lymphocytes altering their expression of the immune response at the cellular level.[24] In adrenal incompetency, Pred acts at steroid ligands.

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Prednisolone is present in the plasma in an excessive amount of free form, and some of the drugs are bound to proteins in the plasma. Although the individual analysis of CPM and Pred, including both the spectrophotometric as well as chromatographic methods has been reported, yet their simultaneous determination has not been sorted out. The combination of these two drugs is generally advised as a remedy for patients with chronic cough and cold.

The aim of the present study was to develop a new method for the quantitative analysis of CPM and Pred, as well as design a pathway for their simultaneous analysis in a single step in active pharmaceutical ingredient (API) and its parental dosage forms.

Materials and Methods

Source of chemicals

Chemicals used during the course of method development and validation of CPM maleate (99%) and Pred (99%) in bulk as well as parenteral dosage form were acetonitrile (HPLC grade), sodium dihydrogen phosphate, and water (MS grade). All these chemicals were purchased from Merck and Sigma Aldrich, and they were of high purity.

Method development

Different trials were made to develop a method for the quantitative determination of CPM maleate and Pred. The guidelines as described in British Pharmacopeia were used. Optimizations in the flow rate and composition of the mobile phase were done to get the optimum results of CPM maleate and Pred in active pharmaceutical ingredients and pharmaceutical dosage form. Different compositions of the mobile phase and flow rate were tested while keeping other parameters such as injection volume, temperature, detection wavelength, and oven temperature constant.

Mobile phase and solution preparation

A solution of 0.008 M sodium dihydrogen phosphate (NaH_2PO_4) was prepared as a buffer. In a 500 mL volumetric flask, 0.5 g sodium dihydrogen phosphate was weighed and dissolved in 100 mL HPLC grade distilled water. The solution was completely dissolved by mechanical shaking and the pH was adjusted to 3.0 using phosphoric acid. For the isocratic mode, the mobile phase was buffer and acetonitrile (50:50 v/v). The mobile phase was filtered using a nylon membrane filter of 0.2 µm pore size and 0.47 mm diameter and sonicated for 15 minutes at room temperature to degas.

HPLC analysis

CPM and Pred standard solutions were analyzed using WATERS HPLC C18 5µm (150 mm. x 4.6 mm, 5 µm) analytical column with mobile phase comprising ACN and buffer (50:50v/v). The detection wavelength was set at 254 nm using a Photodiode Array (PDA) detector and the total run time was in the range of 1 to 10 min.

Method validation

The proposed method was validated according to the guidelines of the International Conference of Harmonization (ICH), British Pharmacopeia (BP), and US Pharmacopeia (USP). Specificity, Linearity, Range, Limit of Detection (LOD), Limit of Quantification (LOQ), Precision, and Bias/Accuracy were studied during this validation process. The following equation was used to calculate uncertainty for method validation authenticity.

$$\text{Uncertainty} = \sqrt{(\%RSD\ of\ Linearity)^2 + (\%RSD\ of\ Bais)^2 + (\%RSD\ of\ Precision)^2}$$

Results and Discussion

Outcome of method development

For the development of the method, different chromatographic conditions were tested. The best result was observed at the buffer of 0.008M NaH_2PO_4 and ACN in the concentration range of 50: 50 v/v. The chromatogram of the simultaneous analysis of CPM (0.40 mg/mL) and Pred (1.0 mg/mL) acquired from the method developed is shown in Figure 1. Retention times (RT) of CPM and Pred were between 1 to 4 min. Samples analyzed in five replicates showed excellent repeatability with RSD in the range of 1.1 and 0.7% for the CPM and Pred, respectively.

Outcome of method validation

An analytical method is a path or way used for the detection or quantification of an analyte in the sample. It gives a detailed description of each step involved in the analysis. Here, the developed method was validated for the parameters which include; linearity, range, specificity, the limit of detection and quantification, precision, and accuracy. The method validation was done according to the guidelines of the International Conference of Harmonization (ICH), British Pharmacopeia (BP), and U.S. Pharmacopeia (USP). Verification was done by estimation of CPM and Pred in the parenteral dosage form.

Linearity

Linearity is usually calculated to check the response of the method in different concentration ranges. The linearity of the method was calculated by analyzing the five concentrations of standard solutions. The calibration curve was drawn between the peak areas of the analyte peaks against the concentration of analyte in the sample. For the entire range of concentrations for both analytes, a linear correlation was observed with a correlation coefficient of 0.9999 (Figure 2).

Limit of detection and limit of quantification

The detection limit (LOD) of an analytical procedure is the minimum amount of the analyte which can be detected by using that method. LOD of the analytical method can be calculated by comparing the signal of the analyte and noise of the baseline by analyzing the lowest amount of analyte.[25] In statistical terms, LOD may be estimated by calculating the slope of the calibration curve and standard deviation of the replicates of the lowest amount of analyte. The obtained LODs for CPM and Pred were 85 and 32 µg/mL, respectively. Similarly, the quantification limit (LOQ) of the analytical procedure was also calculated. According to the international guideline (ICH Guidelines for Method Validation), the signal-to-noise ratio of LOQ should be 10:1. Calculated LOQs for CPM and Pred were 275 and 108 µg/mL, respectively.

Repeatability

Repeatability is the degree of closeness of results obtained by analyzing the number of replicates of the homogeneous samples under the same conditions. The repeatability of the developed method was calculated by preparing the concentrations of standard solutions of Pred and CPM. The CPM and Pred standards were replicated six times with values of 0.4 and 0.1 mg/mL were analyzed. RSD values obtained were 1.1 and 0.7%, respectively.

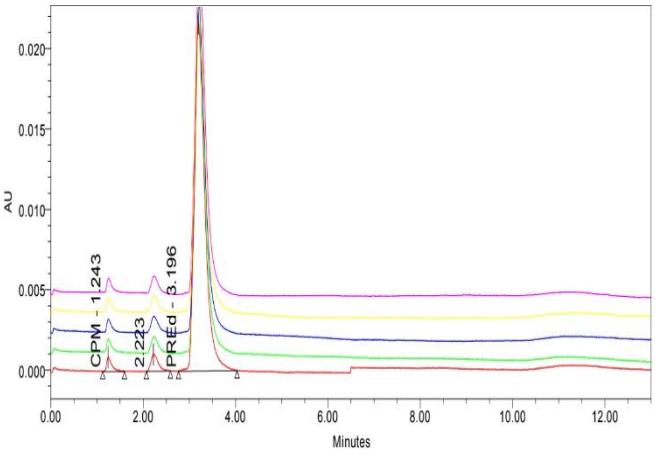

Figure 1: HPLC-PDA chromatogram of chlorpheniramine and prednisolone obtained at 0.008M NaH_2PO_4 and ACN (50:50v/v).

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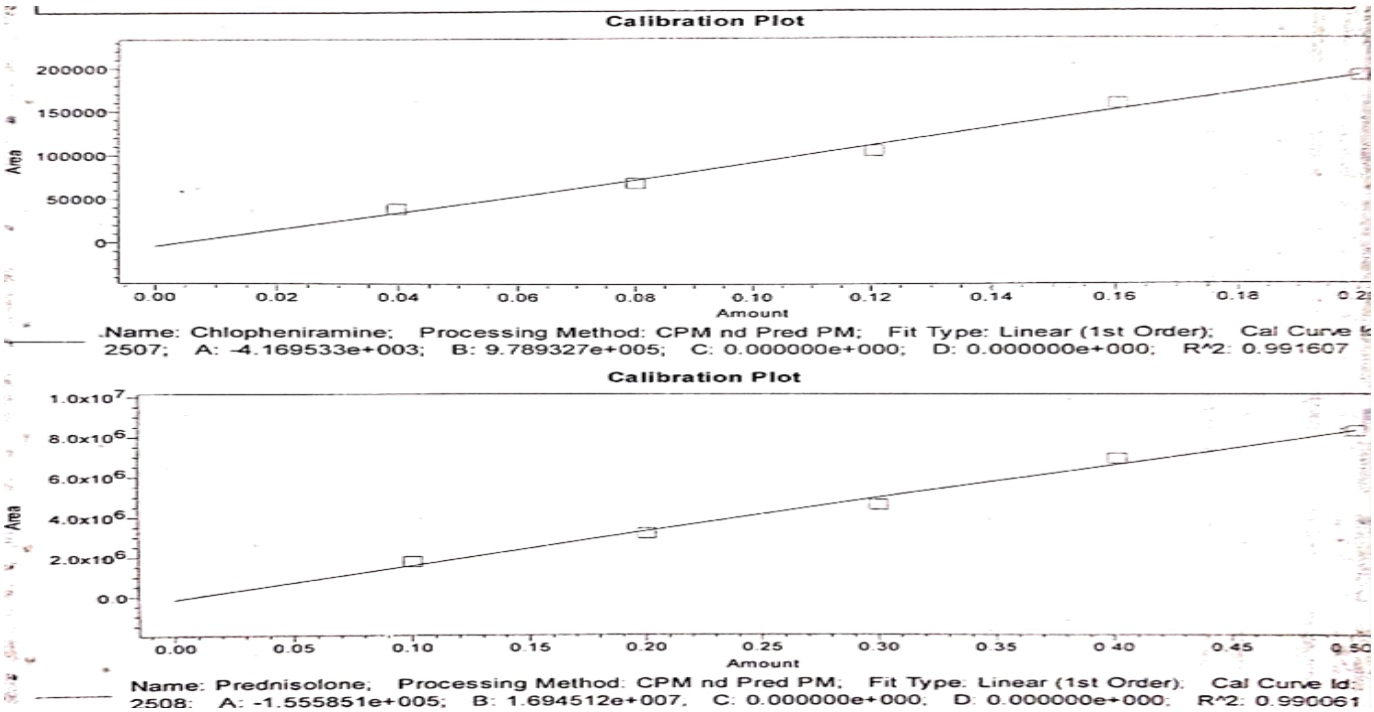

Figure 2: Linearity of the developed method for chlorpheniramine and prednisolone.

The value of relative standardization should be less than 2 for the developed method according to international guidelines, such as the International Conference of Harmonization Guidelines for Method Validation (Q2) and international pharmacopeias (BP and USP). These results indicate that the developed method is repeatable under optimized conditions.

Intermediate precision

According to the ICH guidelines for method validation, intermediate precision of the analytical method is an ability of a method to produce precise results either by analyzing the sample on different equipment, using a different analyte, or analyzing the sample on different days. Here intermediate precision of the developed method was calculated by using 0.4 mg/mL concentration solution of CPM and 0.1 mg/mL of Pred. On two different days, six duplicates of produced analyte standards were examined, and the findings are reported in Table 1. According to the international guidelines and pharmacopeias, the value of the square root of Σ $(\%RSD)^2$ on different days should be less than 2. The devised HPLC-based technique yielded Σ $(\%\ RSD)^2$ values of 1.4 and 1.2 for CPM and Pred respectively.

Accuracy

The accuracy was calculated by spiking 50, 100, and 150% amount of the targeted concentration of the analyte. According to the ICH guidelines for method validation, the accuracy of the analytical procedure should be calculated for at least nine concentrations, particularly three replicates of three different concentrations which cover the entire range of the analyte. In this study, targeted concentration of the analyte was calculated from three replicates of 0.1 mg/mL standard solutions of Pred and 0.4 mg/mL of CPM. Solutions with three different concentrations were prepared by spiking 50, 100, and 150% to the amount of targeted concentration in the sample. Each concentration of the analyte with three replicates were analyzed and the results obtained indicated that both samples showed a recovery from 98 to 100 % as presented in Figure 3.

Uncertainty of the method

Uncertainty of the method should be in the reference limits, otherwise, the method validation is considered null and void. The lower the value of uncertainty, the higher the authenticity of the results. The combined uncertainty of the results was calculated by taking the square root of the sum of the squares of the %RSD of all the validation parameters. In this study, the expanded uncertainty, which is twice the combined uncertainty, was also estimated (Table 2).

Method verification

Method after validation studies is applied to analyze parenteral dosage form for qualitative and quantitative studies of Pred and CPM using a brand name as Pred CPM injection. The method was applied successfully for the Pred CPM injection manufactured by Nova Med Pharma. The method responded well and there was no interference by excipients. The results of dosage forms are shown in Figure 4. The method was verified by using Pred CPM injection (1 mg/mL + 0.4 mg/mL). The HPLC chromatogram shows that the approach may successfully detect both analytes and excipients in commercially available products.

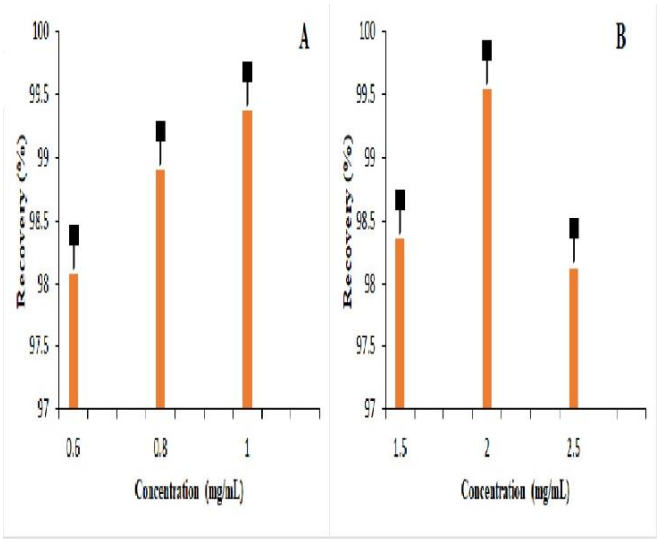

Figure 3: Recovery analysis of (A) CPM and (B) Prednisolone by the developed HPLC method.

Table 1: Calculation of intermediate precision of chlorpheniramine and prednisolone

Replicates	Day One	Day Two	Day One	Day Two
	CPM (0.4 mg/mL)		PRED (1 mg/mL)	
Replicate 1	15474	166584	738796	738279
Replicate 2	15784	164589	737761	720545
Replicate 3	15727	166872	733817	732393
Replicate 4	15686	164825	730924	737935
Replicate 5	15363	167892	725986	736258
Mean	15606.8	166152	733456.8	733082
SD	179.7184	1405.592	5229.504	7387.464
%RSD	1.15	0.8	0.51	1.007
$(\%RSD)^2$	1.32	0.71	0.508	1.01
$\sum(\%RSD)^2$	2.044		1.52	
SQRT of Sum	1.4299		1.2	

*SQRT = Square root; SQRT of Sum = Intermediate Precision; Chlorpheniramine CPM; Prednisolone PRED

Table 2: Uncertainty calculations of the proposed HPLC based method for chlorpheniramine and prednisolone analysis.

Parameter	%RSD of CPM	Square of %RSD of CPM	% RSD of Pred	Square of % RSD of Pred
Linearity	1.05633	1.11584	1.07219	1.1495
Repeatability	1.1	1.21	0.7	0.49
Intermediate Precision	0.8	0.64	1.2	1.44
Practical Bias	0.038029527	0.001446	0.0470914	0.0022175
Sum of Square	1.7	-	-	3.08
Combined uncertainty	1.3	-	-	1.73
Expanded Uncertainty	2.607	-	-	3.46

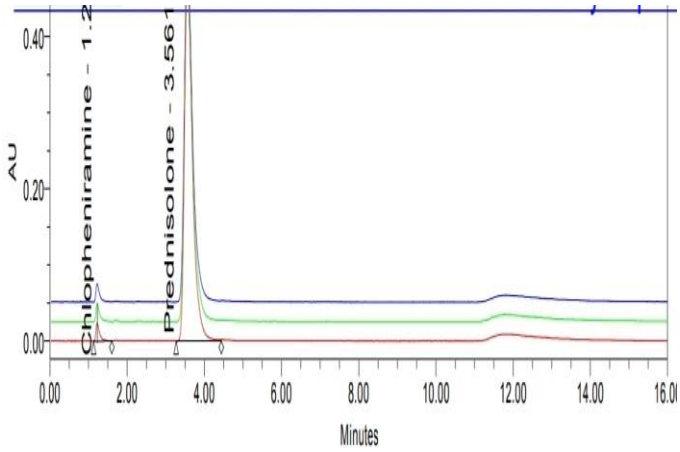

Figure 4: HPLC chromatogram of the sample prednisolone-chlorpheniramine injection. C18 (5µm x 150 mm 4.6 mm) HPLC column; mobile phase 50:50 v/v acetonitrile: 0.008M NaH_2PO_4.

Conclusion

The obtained results revealed that developed method was accurate, rapid, linear, and precise. Validation results of the method proves that it is according defined guidelines of active pharmaceutical ingredients (API). The R^2 values of 0.991607 for CPM and 0.990061 for PRED verified its linearity. RSD value proved its precision according to ICH and pharmacopeia. Outcomes of LOD and LOQ was in range. Accuracy parameters for CPM and PRED are within range of 98 to 99%. Furthermore, system suitability method is reproducible at different days and time. The developed method has successfully applicable for simultaneous determination of PRED and CPM in bulk and parenteral dosage form.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Yamauchi K and Ogasawara M. The role of histamine in the pathophysiology of asthma and the clinical efficacy of antihistamines in asthma therapy. Int J Mol Sci. 2019; 20(7):1733.
2. Mandola A, Nozawa A, Eiwegger T. Histamine, histamine receptors, and anti-histamines in the context of allergic responses. Lymph J. 2019; 6(2):35-51.
3. Durante M, Sgambellone S, Lanzi C, Nardini P, Pini A, Moroni F, Masini E, Lucarini L. Effects of PARP-1 deficiency and histamine H4 receptor inhibition in an

inflammatory model of lung fibrosis in mice. Front Pharm. 2019; 10:525.
4. Bolam JP and Ellender TJ. Histamine and the striatum. Neuropharm. 2016; 106:74-84.
5. Sadek B, Saad A, Sadeq A, Jalal F, Stark H. Histamine H3 receptor as a potential target for cognitive symptoms in neuropsychiatric diseases. Behav Brain Res. 2016; 312:415-430.
6. Arrang JM, Garbarg M, Schwartz JC. Auto-inhibition of brain histamine release mediated by a novel class (H3) of histamine receptor. J Nat. 1983; 302(5911):832.
7. Cox JH, Seri S, Cavanna AE. Safety and efficacy of aripiprazole for the treatment of pediatric Tourette syndrome and other chronic tic disorders. Pedia Helth Med Ther. 2016; 7:57-64.
8. Takeshita K, Bacon KB, Gantner F. Critical role of L-selectin and histamine H4 receptor in zymosan-induced neutrophil recruitment from the bone marrow: Comparison with carrageenan. J Pharm Exper Ther. 2004; 310(1):272-280.
9. Salem A, Rozov S, Al-Samadi A, Stegajev V, Listyarifah D, Kouri VP. Histamine metabolism and transport are deranged in human keratinocytes in oral lichen planus. Br J Derm. 2017; 176(5):1213-1223.
10. Dunford PJ, Williams KN, Desai PJ, Karlsson L, McQueen D, Thurmond RL. Histamine H4 receptor antagonists are superior to traditional antihistamines in the attenuation of experimental pruritus. J Allerg Clin Immunol. 2007; 119(1):176-183.
11. Van Ganse E, Kaufman L, Derde MP, Yernault JC, Delaunois L, Vincken W. Effects of antihistamines in adult asthma: a meta-analysis of clinical trials. Eur Resp J. 1997; 10(10):2216-2224.
12. Mendelson WB, Roth T, Cassella J, Roehrs T, Walsh JK, Woods JH. The treatment of chronic insomnia: drug indications, chronic use and abuse liability. Summary of a 2001 new clinical drug evaluation unit meeting symposium. Sleep Med Rev. 2004; 8(1):7-17.
13. Csiernik R. The Essential Guide to Psychoactive Drugs in Canada: A Resource for Counselling Professionals, 2nd Edition; Canad Schol' Press. 2019; 7-9p.
14. Gami-Yilinkou R, Nasal A, Kaliszan R. Application of chemometrically processed chromatographic data for pharmacologically relevant classification of antihistamine drugs. J Chrom A. 1993; 633(1-2):57-63.
15. Pearlman DS. Antihistamines: pharmacology and clinical use. J Drugs. 1976; 12(4):258-273.
16. Mescheder A, Karrasch M, inventors; Medigene AG, assignee. Method of administering a cationic liposomal preparation. United States patent US 9,233,094. 2016; 5-14.
17. Gray SL, Anderson ML, Dublin S, Hanlon JT, Hubbard R, Walker R. Cumulative use of strong anticholinergics and incident dementia: a prospective cohort study. JAMA Int Med. 2015; 175(3):401-407.
18. Nebreda AD, Zappia CD, González AR, Sahores A, Sosa M, Burghi V, Monczor F, Davio C, Fernández N, Shayo C. Involvement of histamine H1 and H2 receptor inverse agonists in receptor's crossregulation. Eur J Pharm. 2019; 847:42-52.
19. Wang T, Fu H, Kaminga AC, Li Z, Guo G, Chen L, Li Q. Prevalence of depression or depressive symptoms among people living with HIV/AIDS in China: a systematic review and meta-analysis. BMC Psy. 2018; 18(1):1-4.
20. Simons FER and Simons KJ. Histamine and H1-antihistamines: Celebrating a century of progress. J Allerg Clin Immunol. 2011; 128(6):1139-50.
21. Schempp CM, Schauer F, Huhn CK, Venhoff N, Finzel S. Skin inflammation associated with arthritis, synovitis and enthesitis. Part 2: rheumatoid arthritis, reactive arthritis, Reiter's syndrome, Lyme borreliosis, dermatomyositis and lupus erythematosus. JDDG. J der Deut Derm Gese. 2019; 17(2):167-181.
22. Simons FER. Advances in H1-antihistamines. New Eng J Med. 2004; 351(21): 2203-2217.
23. Stahn C, Löwenberg M, Hommes DW, Buttgereit F. Molecular mechanisms of glucocorticoid action and selective glucocorticoid receptor agonists. Mol Cell Endocrinol. 2007; 275(1-2):71-78.
24. Fu XQ, Cai JY, Li MJ. Prednisone may rebuild the immunologic homeostasis: Alteration of Th17 and Treg cells in the lymphocytes from rats' spleens after treated with prednisone-containing serum. Mol Gene Geno Med. 2019; 7(7):e00800.
25. Veeraswami B and Naveen V. Development and validation of rp-hplc method for the estimation of dolutegravir and rilpivirine in bulk and pharmaceutical dosage form and its application to rat plasma. Dev. 2019; 12(2):267-271