CHAPTER 7
SPECTROPHOTOMETRIC DETERMINATION OF FEXOFENADINE HYDROCHLORIDE AND BETAMETHASONE IN PHARMACEUTICALS

SECTION 1

SPECTROPHOTOMETRIC DETERMINATION OF FEXOFENADINE HYDROCHLORIDE IN PHARMACEUTICALS

SECTION II

SPECTROPHOTOMETRIC DETERMINATION OF BETAMETHASONE IN PHARMACEUTICALS
SECTION 1
SPECTROPHOTOMETRIC DETERMINATION OF FEXOFENADINE HYDROCHLORIDE IN PHARMACEUTICALS

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7.1 INTRODUCTION

Antihistamines are H1-receptor antagonists widely used in human and veterinary medicine to provide symptomatic relief of allergic signs caused by histamine release, including pruritus and anaphylactic reactions. They are also commonly used as sedative and antiemetics [1]. Antihistamines can be divided into first and second generation (also called non-sedating) agents. First generation antihistamines are small lipophilic molecules, so they may cause adverse effects because of their cholinergic activity and their ability to cross the blood-brain barrier. Second generation antihistamines are more lipophobic than first generation antihistamines and are thought to lack central nervous system and cholinergic effects when given at therapeutic doses [2]. Fexofenadine hydrochloride, the active ingredient of Allegra and Telfast, is a second-generation histamine H1-receptor antagonist with the chemical name \( \alpha,\alpha -\text{dimethyl}-4-[1\text{-hydroxy}-4-[4-(\text{hydroxydiphenyl- methyl})-1\text{-piperidinyl}]\text{butyl}]\text{-benzene acetic acid.} \) Fexofenadine is used as the hydrochloride salt in the symptomatic relief of allergic conditions including seasonal allergic rhinitis and urticaria [3, 4]. The probability that cardiotoxic side effects occur in connection with fexofenadine is assessed as being extremely low. Since metabolism is not effected by cytochrome P-450 no interaction with other drug is assumed. Fexofenadine does not have a negative effect on the psychomotor efficiency and it shows a high degree of daily-life suitability. The substance has anti-inflammatory characteristics which presents a modern approach to allergy therapy. Besides, fexofenadine may prove a safer alternative in the treatment of asthma [5] and atopic dermatitis [6] and is rapidly absorbed with a long duration of action, making it suitable for once daily administration. Thus, fexofenadine fulfils the essential and desirable characteristics of an ideal antihistamine, being responsible for the improvement in quality of life of the patients with allergic diseases [7]. Fexofenadine hydrochloride is rapidly absorbed after oral administration with peak plasma concentrations being reached in 2–3 h. It is about 60–70% bound to plasma proteins. Elimination half-life is about 14 h [3,4]. It is having the capability to exist in zwitter-ionic form, it cannot pass through blood–brain barrier and therefore does not cause sedation [8,9]. Fexofenadine hydrochloride is official in the USP [10]. Moreover, the literature presents few methods related to the quality control of fexofenadine, mainly in its pharmaceutical dosage forms.
The older antihistaminic agent terfenadine was found to metabolize into the related carboxylic acid, fexofenadine. Fexofenadine was found to retain all of the biological activity of its parent while giving fewer adverse reactions in patients, so terfenadine was replaced in the market by its metabolite [11]. Fexofenadine was originally synthesized in 1993 by Massachusetts-based biotechnology company Sepracor, which then sold the development rights to Hoechst Marion Roussel (now part of Sanofi-Aventis), and was later approved by the Food and Drug Administration (FDA) in 1996. Fexofenadine may be synthesized from piperidine-4-carboxylate ester and 4-bromophenylacetonitrile [11].

To produce the piperidine piece, two phenyl groups are first introduced using a Grignard reaction on the ester, giving a tertiary alcohol. The amine group is then alkylated with a protected aldehyde, then the aldehyde is recovered by deprotection with acid. The remaining piece of the molecule is produced by a double alkylation by iodomethane of the carbanion derived from the nitrile. The nitrile group is then hydrolyzed to a carboxylic acid. The aryl bromide is then lithiated to produce the organolithium compound, which can be coupled with the aldehyde piece to give fexofenadine.

Reports of fexofenadine overdose are infrequent, and because of this, the effects are not well established. No deaths occurred in testing on mice, at 5000 mg kg\(^{-1}\), which is 110 times the maximum recommended dose for an adult human. Further research shows no deaths in rats at the same concentration, which equates four hundred times the recommended dose in an adult human. Research on humans ranges from a single 800 mg dose, to a twice-daily 690 mg dose for a month, with no clinically significant adverse effects, when compared to a placebo. The dissolution test has emerged as a valuable quality control tool to assess batch-to-batch product release performance and to assure the physiological availability of the drug [12]. Its significance is based on the fact that for a drug to be absorbed and available on the systemic circulation, it must previously be solubilized [13]. The low cost and ease of operation make the spectrophotometric method highly desirable alternatives for the assay of fexofenadine hydrochloride in pharmaceuticals. For this reason and as a result of therapeutic importance of fexofenadine hydrochloride, it was of interest to investigate the application of
chloramine-T as a spectrophotometric reagent for the assay of fexofenadine hydrochloride.

### 7.2 ANALYTICAL CHEMISTRY

Several methods have been reported for the determination of fexofenadine hydrochloride. Fexofenadine has been determined in biological fluids by HPLC with mass spectrometry detection [14], ionspray tandem mass spectrometry detection [15,16], and fluorescence detection [17]. The quantitation of fexofenadine in pharmaceutical dosage forms was realized using spectrophotometric methods [18,19], HPLC methods with ultraviolet detection [20,21], potentiometric titration using membrane electrode [22]. In spite of its importance in the therapy of allergic diseases, there is just one report concerning the stability of fexofenadine [23] but the isolation and characterization of its degradation products was not performed.

Sakalgaonkar et al. reported a high-performance liquid chromatographic method for resolution of the structural isomers of fexofenadine hydrochloride in the bulk drug [24]. The isomers were resolved to baseline on a reversed-phase ODS column with pH 3 aqueous buffer-acetonitrile 60:40 containing 5 g L⁻¹  β-cyclodextrin as mobile phase additive. The aqueous buffer was prepared by dissolving 6.8 g KH₂PO₄ in 1000 mL water and adjusting to the pH 3.0 with orthophosphoric acid. Resolution for the structural isomers was not less than 3.0. The calibration plot was indicative of an excellent linear relationship between response and concentration over the range 0.025-3.750 ppm for the meta isomer. The limits of detection and quantification for the meta isomer were 0.003 and 0.009 ppm, respectively, for an injection volume of 20 μL. Recovery of the meta isomer from bulk drug samples of fexofenadine hydrochloride was 98.43, 96.52, and 97.72 % for addition of 0.5, 0.6 and 0.7%, respectively. The analytical solution was stable for 48 h.

Karakus et al. described the determination of fexofenadine with pseudoephedrine in binary pharmaceutical dosage forms [25]. The chromatographic separation of pseudoephedrine hydrochloride, fexofenadine hydrochloride and cetirizine dihydrochloride was achieved on a Zorbax C8 (150 mm × 4.6 mm; 5 μm particle size) column using UV detection at 218 and 222 nm.
The retention times were 1.099, 2.714 and 3.808 min for pseudoephedrine hydrochloride, fexofenadine hydrochloride and cetirizine dihydrochloride respectively. The proposed method provided linear responses within the concentration ranges 30.0-240.0 and 1.25-10.0 μg mL⁻¹ with limit of detection values of 1.75 and 0.10 μg mL⁻¹ for pseudoephedrine hydrochloride and fexofenadine hydrochloride respectively. Linearity range for pseudoephedrine hydrochloride-fexofenadine hydrochloride binary mixtures were 10.0-80.0 and 5.0-40 μg mL⁻¹ with limit of detection values of 0.75 and 0.27 μg mL⁻¹.

Pathak et al. described a simple and rapid high performance liquid chromatographic method with fluorescence detection for the estimation of fexofenadine in rat plasma—application to preclinical pharmacokinetics [26]. The chromatographic separation was achieved using a supelco C₁₈-DB (250 mm x 4.6 mm I.D./5 μm particle size) column with mobile phase comprising of ammonium acetate buffer and acetonitrile (63:37, v/v), delivered isocratically at a flow rate of 1.0 mL min⁻¹. Diphenhydramine was used as an internal standard. The statistical evaluation of the method was examined and the method was found to be precise and accurate with a linearity range of 1.0–500.0 ng mL⁻¹ (r > 0.9980). The intra- and inter-day precision studies showed good reproducibility with coefficients of variation less than 12.26%. The photostability of the antihistamine fexofenadine hydrochloride was described [27]. The stress studies revealed the photostability of the drug as the most adverse stability factor. The main photodegradation products were isolated and its structures were elucidated by NMR and mass spectrometry techniques.

An alternative method for the determination of fexofenadine and pseudoephedrine in their combined tablet formulation was developed [28], employing the partial least squares analysis of spectral data of the analytes in their pharmaceutical association. A full-factorially designed set of 16 synthetic samples was employed for calibration purposes. The described method was linear for both analytes, over the range 160.6-301.2 mg L⁻¹ for fexofenadine (R² = 0.9993) and between 325.6 mg L⁻¹ and 610.5 mg L⁻¹ for pseudoephedrine (R² = 0.9992). It was accurate, exhibiting 99.8% and 99.9% drug recoveries for fexofenadine and pseudoephedrine, respectively (N =9), while in the intermediate precision
experiment relative standard deviations were 1.4% for fexofenadine and 1.2% for pseudoephedrine. Oliveira et al. presented a simple and reliable HPLC analysis of fexofenadine hydrochloride in tablets and its application to dissolution studies [29]. Mobile phase:triethylamine phosphate 1%, pH 3.2:acetonitrile(ACN): methanol (50:30:20), 210 nm detection, C18 Phenomenex® column. The method was validated regarding accuracy/precision (RSD < 1%), linearity ($r^2 = 0.9999$), and robustness. The method was applied to the determination of the drug in commercial tablet preparations and proved to be fast and reliable for quantification and it was also used for the comparison of dissolution profiles of fexofenadine hydrochloride tablets. Breier et al. presented the kinetics of photodegradation of the antihistamine fexofenadine hydrochloride using a stability-indicating high performance liquid chromatography [30]. The degradation was carried out in methanol and in water solutions, prepared from coated tablets, in quartz cells under UV light at 254 nm. The kinetics parameters of order of reaction and the rate constants of the degradation were determined for both solvents. The degradation process of fexofenadine hydrochloride in solutions can be described by second-order kinetics under the experimental conditions used in this study.

Suresh Kumar et al. reported a method for the determination of fexofenadine hydrochloride in bulk and pharmaceutical dosage forms [31]. The method was based on the chloroform-extractable pale yellow color complex formed by the reaction of fexofenadine with bromothymol blue at pH 2.6. The chromogen can be estimated at 412 nm against the reagent blank. This method obeyed Beer’s law in the concentration range of 10.0-50.0 μg mL$^{-1}$ of drug. The optimum reaction conditions and other analytical parameters were also evaluated. Uno et al. reported lack of dose-dependent effects of itraconazole on the pharmacokinetic interaction with fexofenadine [32]. Brier et al. reported the development and validation of dissolution tests for fexofenadine hydrochloride capsules and coated tablets [33]. Fexofenadine hydrochloride capsules, products A and B, and coated tablets, products A, B and C were evaluated. The parameters of difference factor, similar factor, and dissolution efficacy were employed. Optimal conditions to carry out the dissolution tests were 900 mL of 0.01 M hydrochloric acid as dissolution medium, basket at 100 rotation per min (rpm) stirring speed for capsules and paddle at 75 rpm for tablets. The dissolution profiles for tablets
products A, B, and C and for capsules products A and B were not similar. Thus, the developed and validated dissolution tests satisfactorily describes the time-course of the drug release. The obtained results provided adequate dissolution profiles. The HPLC method was validated to quantify fexofenadine capsules and coated tablets from the dissolution tests.

Golcu et al. reported anodic voltammetric behavior and determination of antihistaminic agent: Fexofenadine hydrochloride [34]. The electrochemical oxidation of fexofenadine hydrochloride was investigated by cyclic, linear sweep, differential pulse and square wave voltammetry using glassy carbon electrode. The oxidation of fexofenadine hydrochloride was irreversible and exhibited diffusion-controlled process depending on pH. The dependence of intensities of currents and potentials on pH, concentration, scan rate, nature of the buffer were investigated. The linear response was obtained in supporting electrolyte in the ranges of $1.0 \times 10^{-6}$ - $2.0 \times 10^{-4}$ M with a detection limit of $6.6 \times 10^{-9}$ M and $5.76 \times 10^{-8}$ M and in serum samples in the ranges of $2.0 \times 10^{-6}$ - $1.0 \times 10^{-4}$ M with a detection limit of $8.08 \times 10^{-8}$ M and $4.97 \times 10^{-8}$ M for differential pulse and square wave voltammetric techniques, respectively. Only square wave voltammetric technique can be applied to the urine samples, and the linearity was obtained in the ranges of $2.0 \times 10^{-6}$ - $1.0 \times 10^{-4}$ M with a detection limit of $2.00 \times 10^{-7}$ M.

Shimizu et al. reported the contribution of oatp (organic anion-transporting polypeptide) family transporters to the hepatic uptake of fexofenadine in humans [35]. The development and validation of a new, simple, fast and sensitive liquid chromatographic method for the determination of the antihistamine fexofenadine was described [36]. Through the evaluation of the analytical parameters, it was shown that the method was linear ($r = 0.9999$) at concentrations ranging from 20.0 to 80.0 $\mu$g mL$^{-1}$, precise (intraday relative standard deviation [RSD] values = 0.85, 0.40, and 0.81%; interday RSD = 0.77 %), accurate (mean recovery, 99.05%), specific, and robust. The detection and quantitation limits were 0.341 and 1.033 $\mu$g mL$^{-1}$ respectively.

Abbas et al. described a novel membrane sensor for histamine H1-receptor antagonist “fexofenadine” [37]. The construction and general performance of thirteen new polymeric membrane sensors for the determination of fexofenadine
hydrochloride based on its ion exchange with reineckate, tetraphenylborate and tetraiodomercurate were studied. The novel sensor based on reineckate exchanger showed a stable, potentiometric response for fexofenadine in the concentration range of $1 \times 10^{-2}$ - $2.5 \times 10^{-6}$ M at 25°C that was independent of pH in the range of 2.0 - 4.5. The sensor possesses a Nernstian cationic slope of $62.3 \pm 0.7$ mV/concentration decade and a lower detection limit of $1.3 \times 10^{-6}$ M with a fast response time of 20 - 40 s. There was negligible interference from almost all studied cations, anions, and pharmaceutical excipients, however, citizine that had a structure homologous to that of fexofenadine was found to interfere.

Radhakrishna and Reddy developed a simple reversed phase liquid chromatographic method and subsequently validated for the determination of fexofenadine hydrochloride and its related compounds A and B [38]. The method utilizes a C8 column for the separation and determination of meta-isomer (related compound B). The separation was achieved using an Eclipse XDB C8, 5µm, 4.6×150 mm column and a mobile phase comprising 1% triethylamine phosphate (pH 3.7), acetonitrile and methanol in the ratio 60:20:20 (v/v/v). 5-Methyl 2-nitrophenol has been used as internal standard for the purpose of quantitation of fexofenadine. The described method was linear over a range of 0.7-18.7 µg mL\(^{-1}\) for related compounds A and B and 60.0-750.0 µg mL\(^{-1}\) for assay of fexofenadine. The relative standard deviation (n=3) was 0.5% for the drug and 3.4% for related compounds. The intermediate precision was 0.79% (n=9) for assay and 5.16% (n=9) for related impurities. The mean recovery of both the related compounds were in the range of 94-103%. Limits of detection and quantification for the related compounds A and B were 0.18, 0.12 and 0.56, 0.48 µg mL\(^{-1}\), respectively.

Hofmann et al. reported a method to determine fexofenadine in human plasma and urine by HPLC–electrospray mass spectrometry with MDL 026042 as internal standard [39]. Extraction was carried out on C\(_{18}\) solid-phase extraction cartridges. The mass spectrometer was operated in the selected ion monitoring mode using the respective MH\(^+\) ions, \(m/z\) 502.3 for fexofenadine and \(m/z\) 530.3 for the internal standard. The limit of quantification achieved with this method was 0.5 ng mL\(^{-1}\) in plasma and 1.0 ng in 50 µL of urine. Zarapkar et al. reported a simple, fast and precise reverse phase high performance liquid chromatographic method
for the simultaneous determination of fexofenadine hydrochloride and pseudoephedrine sulfate in its tablet form [40]. A 5μ Inertsil C8 column 25 cm in isocratic mode, with mobile phase 0.025 M ortho - phosphoric acid and acetonitrile (60:40 v/v) pH adjusted to 3.5 with triethylamine were used. The flow rate was 1.0 mL min⁻¹ and effluent was monitored at 215 nm. Methyl Paraben was used as an internal standard.

Rajput and Parekh reported three simple analytical methods based on derivative spectroscopy, difference spectrophotometry and colorimetry for the estimation of fexofenadine hydrochloride in bulk drug and in its film coated tablet formulation [41]. The maxima and minima of second derivative spectrum curve of fexofenadine hydrochloride were obtained at 233 and 222 nm. The calibration curve was linear in the concentration range of 50.0-20.0 μg mL⁻¹. The difference spectrum was generated between 200-300 nm. The maxima and minima were obtained at 223 nm and 208.5 nm respectively. In colorimetric method, fexofenadine was treated with potassium iodide in acidic medium to get a yellow chromogen which was extracted in chloroform and determined at 363 nm. Beer’s law was obeyed in the concentration range of 20.0-70.0 μg of fexofenadine per mL.

The present work describes a novel, sensitive and selective reaction for the assay of fexofenadine hydrochloride in bulk drug and tablet dosage form using chloramine-T and two dyes malachite green and xylene cyanol FF. Spectrophotometric method entail the addition of a known excess of chloramine-T to fexofenadine hydrochloride in hydrochloric acid medium followed by the determination of residual oxidant by reacting it with a fixed amount of malachite green, measuring the absorbance at 615 nm or xylene cyanol FF, measuring the absorbance 612 nm. The proposed method exhibit useful analytical characteristics such as sensitivity, accuracy and precision for the assay of fexofenadine hydrochloride in pure and tablet dosage forms. Comparison of the spectrophotometric method with the earlier methods are shown in table 7.
7.3 APPARATUS
7.3.1 Spectrophotometer
A SHIMADZU (Model No: UV-2550) UV-Visible spectrophotometer with 1 cm matching quartz cells were used for the absorbance measurements.

7.4 REAGENTS AND SOLUTIONS
All reagents used were of analytical reagent grade and distilled water was used for the preparation of all solutions. A 1000 µg mL\(^{-1}\) standard drug solution of fexofenadine hydrochloride was prepared in 50% ethanol and was made up to the mark with distilled water. The stock solution was diluted appropriately to get the working concentration. Hydrochloric acid (5M), chloramine –T (CAT) (0.02M), xylene cyanol FF (XFF) (0.05%), malachite green (MAG) (0.05%) were also used.

7.5 PROCEDURES
7.5.1 Using Malachite Green as Reagent
An aliquot of sample solution containing 0.20-4.00 µg mL\(^{-1}\) of fexofenadine hydrochloride was transferred in to a series of 10 mL calibrated flasks by means of a micro burette. Then, 2 mL of 5M HCl was added followed by 1mL of chloramine T solution. The contents were shaken well and were set aside for 15 minutes with occasional shaking. Then, 0.5 mL of malachite green was added to each flask, and the volume was adjusted up to the mark with distilled water and mixed well. The absorbance of each solution was measured at 615 nm against the corresponding reagent blank.

7.5.2 Using Xylene Cyanol FF as Reagent
An aliquot of sample solution 0.60-4.00 µg mL\(^{-1}\) of fexofenadine hydrochloride was transferred in to a series of 10 mL calibrated flasks by means of a micro burette. Then, 2 mL of 5M HCl was added followed by 1mL of chloramine T solution. The contents were shaken well and were set aside for 15 minutes with occasional shaking. Then, 0.5 mL of xylene cyanol FF was added to each flask, and the volume was adjusted up to the mark with distilled water and mixed well. The absorbance of each solution was measured at 612 nm against the corresponding reagent blank.
7.5.3 Analysis of Dosage Forms

Fexofenadine hydrochloride tablet (Aventis Pharma Limited, Ankleshwar, 120mg/tablet) was taken, and the sample stock solution was prepared by grinding the tablet using a mortar and pestle and transferring to a 100 mL volumetric flask by washing with ethanol. The solution was shaken for 30 minutes and filtered through Whatman no.1 filter paper and the clear solution was made up to 100 mL. Known amount of this solution was taken and analyzed for fexofenadine content following the above procedure without any modification. The results are listed in table 7.1A and 7.1B.

7.6 RESULTS AND DISCUSSION

In the present method, two new dyes malachite green and xylene cyanol FF are introduced. The methods are based on the oxidation of fexofenadine hydrochloride by chloramine-T in HCl medium and the reaction is followed by spectrophotometry for quantization purposes. The determinations of fexofenadine hydrochloride are indirect and are based on the determination of surplus chloramine-T after the oxidation reaction of fexofenadine hydrochloride by the later. The method is based on the reaction of surplus chloramine-T with the corresponding dye solution in acidic medium, which bleaches the colored dye solution to colorless leucoform, the decoloration being caused by the oxidative destruction of the dye, which were measured at 615 and 612 nm for malachite green and xylene cyanol FF respectively (fig. VII.A and VII.B and the reaction mechanism are shown in scheme 7.3A and 7.3B.

7.6.1 Optimization of Experimental Conditions

The drug undergoes oxidation to the corresponding ketone according to the reaction scheme given in scheme 7.2A, since the reaction stoichiometry is found to be 1:1. The oxidation is found to be complete and quantitative in 20 -25 minutes. Formation of corresponding ketone is confirmed by performing Borsche’s reagent test. Many dyes are irreversibly destroyed to colorless products in acidic medium by oxidizing agents and this has been exploited for the indirect spectrophotometric determination of fexofenadine hydrochloride. Fexofenadine hydrochloride when added in increasing concentration to a fixed concentration of chloramine T, consumes the later and there will be a concomitant decrease in its concentration. A
concomitant increase in the concentration of dye resulted when a fixed concentration of the dye is added to decreasing concentration of chloramine T. Preliminary experiments are performed to fix the concentration of the dye that could be measured spectrophotometrically and are found to be 0.05% (0.5 mL) and 0.05% (1 mL) for malachite green and xylene cyanol FF respectively. Preliminary investigation shows that hydrochloric acid is better than sulphuric, phosphoric or acetic acid. It is found that the maximum color developed within 20 minutes and remained almost stable for about 2h. The color development is independent of temperature in the range of 30–35°C.

7.6.2 Analytical Data

The adherence to Beer’s law is studied by measuring the absorbance values of solution varying drug concentration. A straight line graph is obtained by plotting absorbance against concentration of fexofenadine hydrochloride. The calibration graph is linear in the range of 0.20-4.00 µg mL⁻¹ of fexofenadine hydrochloride for fexofenadine hydrochloride-malachite green system, 0.60-4.00 µg mL⁻¹ of fexofenadine hydrochloride for fexofenadine hydrochloride-xylene cyanol FF system. The molar absorptivity, Sandell’s sensitivity for fexofenadine hydrochloride-malachite green and fexofenadine hydrochloride-xylene cyanol FF are found to be 4.09×10⁴ L mol⁻¹ cm⁻¹, 0.012 µg cm⁻², 3.07×10⁴ L mol⁻¹ cm⁻¹, 0.016 µg cm⁻² respectively. Slope, intercept and correlation coefficient for fexofenadine hydrochloride-malachite green system is found to be 0.006, 0.045, 0.9986, while that for fexofenadine hydrochloride-xylene cyanol FF system is found to be 0.057, 0.006, 0.9986 respectively. Adherence to Beer’s law for the determination of fexofenadine hydrochloride using malachite green and xylene cyanol FF are shown in fig. 7A and 7B.

7.6.3 Method Validation

To evaluate the accuracy and precision of the methods, pure drug within the working limits are analyzed, each determination being repeated five times. The results are shown in table 7.1C and 7.1D. In order to check the validity of the proposed methods, fexofenadine hydrochloride is determined in some commercial formulations. From the result it was clear that there is close agreement between the results obtained by the proposed methods and the label claim. The results are also
compared statistically by a student’s t-test for accuracy and variance ratio F-test for precision with those of the literature method [31] at 95% confidence level.

7.7 APPLICATIONS

Selectivity can be described as the capability of the method to accurately measure the response of the analysed compound with no interferences originating from sample matrix. High percentage recovery observed with assay samples of pharmaceutical dosage forms, including standard addition experiments, indicate that the proposed method is not affected by interferences from excipients used in formulations. The described method has been extensively validated in terms of specificity, linearity, accuracy, and precision and system suitability. The results of assay compared favorably with the reference method (Table 7.1A and 7.1B).

7.8 CONCLUSIONS

The methods offer linear ranges of applicability, stable colored species and shorter contact times, and are free from either heating or extraction steps. The proposed method has been successfully applied to the determination of fexofenadine hydrochloride in pharmaceutical samples. The obtained results for the determination of fexofenadine hydrochloride indicate the accuracy and precision of the method.
TABLE 7: COMPARISON OF THE SPECTROPHOTOMETRIC METHOD WITH THE EARLIER METHODS

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Range (µg mL(^{-1}))</th>
<th>(\lambda_{\text{max}}) (nm)</th>
<th>Molar absorptivity (L mol(^{-1}) cm(^{-1}))</th>
<th>Method</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bromothymol blue</td>
<td>10.00-50.00</td>
<td>412</td>
<td>2.30×10(^4)</td>
<td>Spectrophotometry</td>
<td>[31]</td>
</tr>
<tr>
<td>Hydrochloric acid</td>
<td>5.00-20.00</td>
<td>233</td>
<td>1.08×10(^4)</td>
<td>Derivative spectroscopic</td>
<td>[41]</td>
</tr>
<tr>
<td>Hydrochloric acid</td>
<td>50.00-80.00</td>
<td>233</td>
<td>2.28×10(^4)</td>
<td>Difference spectroscopic</td>
<td>[41]</td>
</tr>
<tr>
<td>Potassium iodide</td>
<td>20.00-70.00</td>
<td>363</td>
<td>1.15×10(^4)</td>
<td>Colorimetric</td>
<td>[41]</td>
</tr>
<tr>
<td>Proposed method</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Using Malachite green</td>
<td>0.20-4.00</td>
<td>615</td>
<td>4.09×10(^4)</td>
<td>Spectrophotometry</td>
<td></td>
</tr>
<tr>
<td>Using Xylene cyanol FF</td>
<td>0.60-4.00</td>
<td>612</td>
<td>3.07×10(^4)</td>
<td>Spectrophotometry</td>
<td></td>
</tr>
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</table>
TABLE 7.1A: RESULTS OF ASSAY OF FORMULATIONS BY THE PROPOSED METHOD USING MALACHITE GREEN AS A REAGENT

<table>
<thead>
<tr>
<th>Sample</th>
<th>FFH certified (mg)</th>
<th>Found± SD&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Recovery (%)</th>
<th>&lt;sup&gt;a&lt;/sup&gt;t-test</th>
<th>&lt;sup&gt;b&lt;/sup&gt;F-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feofenadine hydrochloride</td>
<td>120.00</td>
<td>120.04±0.04</td>
<td>100.03</td>
<td>2.18</td>
<td>4.00</td>
</tr>
</tbody>
</table>

<sup>a</sup>Mean ± Standard deviation (n=5) [mg/tablet]
<sup>b</sup>Tabulated t-value at 95% confidence level is 2.31
<sup>c</sup>Tabulated F-value at 95% confidence level is 6.39
Fexofenadine hydrochloride tablet- Aventis Pharma Limited, Ankleshwar

TABLE 7.1B: RESULTS OF ASSAY OF FORMULATIONS BY THE PROPOSED METHOD USING XYLENE CYANOL FF AS A REAGENT.

<table>
<thead>
<tr>
<th>Sample</th>
<th>FFH certified (mg)</th>
<th>Found± SD&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Recovery (%)</th>
<th>&lt;sup&gt;a&lt;/sup&gt;t-test</th>
<th>&lt;sup&gt;b&lt;/sup&gt;F-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feofenadine hydrochloride</td>
<td>120.00</td>
<td>119.99±0.07</td>
<td>99.98</td>
<td>0.13</td>
<td>1.30</td>
</tr>
</tbody>
</table>

<sup>a</sup>Mean±Standard deviation (n=5) [mg/tablet]
<sup>b</sup>Tabulated t-value at 95% confidence level is 2.31
<sup>c</sup>Tabulated F-value at 95% confidence level is 6.39
Fexofenadine hydrochloride tablet- Aventis Pharma Limited, Ankleshwar
TABLE 7.1C: EVALUATION OF ACCURACY AND PRECISION
FEXOFENADINE HYDROCHLORIDE (USING MLG AS A REAGENT)

<table>
<thead>
<tr>
<th>Amount taken (µg mL(^{-1}))</th>
<th>Amount found(^a) (µg mL(^{-1}))</th>
<th>RE (%)</th>
<th>SD</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.20</td>
<td>0.201</td>
<td>0.50</td>
<td>0.001</td>
<td>0.49</td>
</tr>
<tr>
<td>0.40</td>
<td>0.404</td>
<td>1.00</td>
<td>0.003</td>
<td>0.74</td>
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<tr>
<td>0.60</td>
<td>0.606</td>
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<td>0.010</td>
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<tr>
<td>0.80</td>
<td>0.807</td>
<td>0.88</td>
<td>0.008</td>
<td>0.99</td>
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<td>1.00</td>
<td>0.982</td>
<td>-1.80</td>
<td>0.010</td>
<td>1.00</td>
</tr>
</tbody>
</table>

\(a\)- Average of five determinations, SD- Standard deviation, RE-Relative error

TABLE 7.1D: EVALUATION OF ACCURACY AND PRECISION
FEXOFENADINE HYDROCHLORIDE (USING XFF AS A REAGENT)

<table>
<thead>
<tr>
<th>Amount taken (µg mL(^{-1}))</th>
<th>Amount found(^a) (µg mL(^{-1}))</th>
<th>RE (%)</th>
<th>SD</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00</td>
<td>1.006</td>
<td>0.60</td>
<td>0.01</td>
<td>0.99</td>
</tr>
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<td>2.026</td>
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<td>3.038</td>
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<td>0.65</td>
</tr>
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<td>4.00</td>
<td>4.032</td>
<td>0.80</td>
<td>0.017</td>
<td>0.42</td>
</tr>
</tbody>
</table>

\(a\)-Average of five determinations, SD- Standard deviation, RE-Relative error
FIGURE VII. 1: ABSORPTION SPECTRUM FOR FEXOFENADINE-HYDROCHLORIDE-MAG SYSTEM

FIGURE VII. 2 – ABSORPTION SPECTRUM FOR FEXOFENADINE HYDROCHLORIDE-XFF SYSTEM
FIGURE 7.A: ADHERENCE TO BEER’S LAW FOR THE DETERMINATION OF FEXOFENADINE HYDROCHLORIDE USING MAG AS A REAGENT

FIGURE 7.B: ADHERENCE TO BEER’S LAW FOR THE DETERMINATION OF FEXOFENADINE HYDROCHLORIDE USING XFF AS A REAGENT
SCHEME 7.2 A: REACTION OF FEXOFENADINE HYDROCHLORIDE WITH CHLORAMINE T IN ACIDIC MEDIUM

\[
\text{SCHEME 7.2 B}
\]

Unreacted CAT/H$^+$

Malachite green (Colored)

Malachite green (Leucoform)

Xylene cyanol FF (Colored)

Xylene cyanol FF (Leucoform)
SECTION II
SPECTROPHOTOMETRIC DETERMINATION OF BETAMETHASONE
IN PHARMACEUTICALS

7.1.1 INTRODUCTION
7.1.2 ANALYTICAL CHEMISTRY
7.1.3 APPARATUS
7.1.4 REAGENTS AND SOLUTIONS
7.1.5 PROCEDURES
7.1.6 RESULTS AND DISCUSSION
7.1.7 APPLICATIONS
7.1.8 CONCLUSIONS
7.1.9 REFERENCES
7.1.1 INTRODUCTION

Betamethasone is a moderately potent glucocorticoid steroid with anti-inflammatory and immunosuppressive properties. Derivatives of betamethasone, such as phosphate, dipropionate and valerate esters, are used commercially in many pharmaceutical formulations including injectables, creams and aerosols. The regioselective opening of 9,llβ-epoxide with hydrogen fluoride to produce fluorohydrin has been the most important synthetic method. Unlike other drugs with these effects, betamethasone does not cause water retention. It is applied as a topical cream, ointment, foam, lotion or gel to treat itching (e.g. from eczema). Betamethasone sodium phosphate is sometimes prescribed as an intramuscular injection (I.M) for itching from various ailments including allergic reactions to poison ivy and similar plants.

In the United States and Canada, betamethasone is mixed with clotrimazole and sold as lotrisone. Betamethasone is a corticosteroid used to stimulate fetal lung maturation, and to decrease the incidence and mortality from intracranial hemorrhage in premature infants. It is also used as a topical cream to relieve skin irritation. The tablet form, available in Japan, eradicates eczematous rash within 24 hours, far more rapidly than the ointment. Betamethasone cream (0.05%) appears effective in treating phimosis in boys, and often averts the need for circumcision. Betamethasone sodium phosphate is used orally and via injection with the same indications as other steroids. Betamethasone dipropionate and salicylic acid can be used as a treatment for local psoriasis. Betamethasone and other synthetic corticosteroids are widely used as anti inflammatoq, anti allergenic and anti-rheumatic agent [42].

7.1.2 ANALYTICAL CHEMISTRY

Owing to their great therapeutic importance and widespread use, several methods have been reported covering the determination of betamethasone [43-45]. Alcoholic solutions of betamethasone and other corticosteroids containing an α-keto group at C-17 reduce tetrazolium salts to form the colored formazan which can be measured spectrophotometrically [46]. This method has been introduced as an official method for the determination of corticosteroids [47,48]. Recently, high
performance liquid chromatography (HPLC) has been used in the United States Pharmacopeia for their determination [49].

Wiedersberg et al. presented pharmacodynamics and dermatoparmacokinetics of betamethasone 17-valerate [50]. Betamethasone 17-valerate was formulated in different vehicles and the drug concentration was adjusted to either (i) equal thermodynamic activity, or (ii) a range of values up to that corresponding to 80% of maximum thermodynamic activity. Vasoconstriction, an accepted and widely used method to determine bioavailability and bioequivalence of topical steroids, was quantified with a chromameter over 24 h post-removal of the formulation. Drug uptake into the stratum corneum was assessed by tape-stripping. The drug at the same thermodynamic activity in different vehicles provoked similar skin blanching responses, while dermatopharmacokinetic technique profiles distinguished between the formulations. Further, skin blanching responses and drug uptake into the stratum corneum clearly depended upon the absolute betamethasone 17-valerate concentration applied.

Zou et al. presented the determination of betamethasone and betamethasone 17-monopropionate in human plasma by liquid chromatography-positive/negative electrospray ionization tandem mass spectrometry [51]. Both compounds were extracted from human plasma with ether-cyclohexane (4:1, v/v) and were separated by HPLC on a Hanbon Lichrospher C18 column with a mobile phase of methanol-water (85:15, v/v) at a flow rate of 0.7 mL min$^{-1}$. Calibration curves were linear over the range of 0.10-50.0 ng mL$^{-1}$ for betamethasone and 0.050-50.0 ng mL$^{-1}$ for betamethasone 17 valerate. The inter-run relative standard deviations were less than 14.4% for betamethasone and 12.3% for betamethasone 17 valerate. The mean plasma extraction recovery for betamethasone and betamethasone 17 valerate were in the ranges of 82.7-85.9% and 83.6-85.3%, respectively. The method was successfully applied to study the pharmacokinetics of a new formulation of betamethasone phosphate/betamethasone dipropionate injection in healthy Chinese volunteers. Song et al. presented a simple and reliable micellar electrokinetic capillary chromatography method for the simultaneous determination of betamethasone and its epimer dexamethasone in human urine and
A three level full factorial experimental design was employed to search for the optimum conditions. The proposed method was validated with respect to stability, precision, linearity and accuracy. Good relationship between peak area ratio and analyte concentration was linear over 30-1,000 µg mL⁻¹ for betamethasone and dexamethasone with correlation coefficients ≥0.9993.

A liquid chromatography-tandem mass spectrometric method was developed and validated for the determination of betamethasone in human plasma [53]. The analyte was isocratically eluted on a Venusil XBP C₈ column (200 mm × 3.9 mm ID, 5 µm) with methanol-water (containing 5 mmol L⁻¹ ammonium formate) (80:20) at a flow rate of 0.4 mL min⁻¹. Ions monitored in the multiple reaction monitoring mode were m/z 393.3 → 355.2 for betamethasone and m/z 361.3 → 343.2 for prednisolone. Betamethasone was extracted from 0.5 mL human plasma with ethyl acetate. The average recovery was 88.24% and the low limit of quantitation was 0.5 ng mL⁻¹. The 3-day validation study demonstrated excellent precision and accuracy across the calibration range of 0.5 - 80.0 ng mL⁻¹. Deng et al. reported liquid chromatography/tandem mass spectrometry for the determination of betamethasone concentration in rabbit plasma [54].

Determination of betamethasone and chloramphenicol in a pharmaceutical preparation using a short monolithic column coupled to a sequential injection system was reported [55]. A short monolithic column coupled with a sequential injection analysis system enabled separation of two compounds in one step. A Chromolith Flash RP-18e, 25 × 4.6 mm column with a 5 mm precolumn (Merck, Germany) and a FIAlab 3000 system (USA) with a 6-port selection valve and 5 mL syringe were used for sequential injection chromatographic separations in this study. The basic validation parameters showed good results: linearity of determination for both compounds including internal standard (propylparaben) >0.999; repeatability of determination in the range 0.8-1.7% at two different concentration levels, and detection limits in the range 0.5-1.0 mg mL⁻¹. The chromatographic resolution between compound peaks was greater than 2.1 and the analysis time was less than 8 min under optimal conditions. Luo et al. presented resolution, quantification and confirmation of betamethasone and dexamethasone in equine plasma by liquid chromatography/tandem mass spectrometry [56].
Analytes were directly extracted from equine plasma by methyl tert-butyl ether. The residues were reconstituted with sample solvent. Liquid chromatography separation of the analytes was performed on a Hypercarb column using acetonitrile/water/formic acid (95:5:0.5, v/v/v) as the mobile phase. Sample screening, quantification and confirmation were performed in multiple reaction monitoring mode. The method was linear over the concentration range of 0.1-75.0 ng mL$^{-1}$ for both analytes. The intra-and inter-day precisions expressed as coefficient of variation for quantification of dexamethasone and betamethasone from 0.1 to 50.0 ng mL$^{-1}$ were less than 7% and the accuracy was in the range of 97-105%.

Wulandari and Indrayanto presented a densitometric method for determination of betamethasone dipropionate and salicylic acid in lotions [57]. The samples were diluted with 96% ethanol and spotted on precoated silica gel TLC plates which were then eluted with ethanol (96%)-toluene-chloroform-glacial acetic acid, 6.0 $\times$ 20 $\times$ 14 $\times$ 0.5 (v/v). Quantitative evaluation was performed by measuring the absorbance reflectance of the betamethasone dipropionate and salicylic acid spots at $\lambda$ = 250 and 310 nm, respectively. HPTLC determination of betamethasone in tablets and its validation was reported [58]. After extraction of the analyte with 96% ethanol, the extracts were spotted on precoated HPTLC silica gel plates, which were then developed with a mixture of chloroform-methanol-water (18: 5: 0.5). Quantitative evaluation was performed by measuring the absorbance reflectance of the analyte spots at 245 nm.

Belgaied described the electrochemical reduction of betamethasone valerate in a pharmaceutical formulation containing neomycin has been carried out in Britton-Robinson buffer (0.04 mol L$^{-1}$) by differential-pulse polarography [59]. Betamethasone valerate exhibits a well-defined irreversible reduction peak at -1.03 V/ref. The influence of pH on the reduction of betamethasone valerate was studied in Britton-Robinson buffer (pH range 1.7-10). A method for the analysis of betamethasone valerate in Britton-Robinson buffer (0.04 mol L$^{-1}$), which allows quantification over the range 3.9$\times$10$^{-6}$-1.1$\times$10$^{-4}$ mol L$^{-1}$, was proposed and successfully applied to the determination of betamethasone valerate in tablets with
mean recovery and relative standard deviation of 100.81% and 0.45%, respectively.

Wang et al. presented the determination of betamethasone disodium phosphate in the in vitro media of PLGA microspheres by high-performance liquid chromatography [60]. In this work, polymeric microspheres loaded with betamethasone disodium phosphate were fabricated to design a sustained-release system. A validated HPLC technique for determination of betamethasone disodium phosphate in vitro media of the polymeric microspheres was developed. The operation conditions were optimized. The analytical column was ZORBAX® Bonus-RP column with 4.6×150 mm ID and a particle size of 5 μm. The mobile phase consisted of acetonitrile-0.01% phosphoric acid water solution (40:60, v/v). The flow rate was 1.0 mL min\(^{-1}\) and injection volume was 50 μL. The elutes were detected at 240 nm. Linearity, repeatability, inter- and intra-assay precision and accuracy of the method were evaluated. The liner range was obtained in a concentrated range of 20-1000 μg mL\(^{-1}\), with a coefficient of correlation \(r=0.999978\). The limit of detection for betamethasone disodium phosphate in the in vitro test samples was 0.25 μg. Recovery of betamethasone disodium phosphate from the in vitro test samples was 99.7±5.2% (mean±SD).

Jin and Wang reported simultaneous HPLC determination of betamethasone sodium phosphate and betamethasone dipropionate in compound betamethasone injection [61]. The analysis was carried on a column of Shim-Pack CLC-ODS (4.6 mm × 150 mm) with a mobile phase of methanol-0.05 mol·L\(^{-1}\) potassium dihydrogen phosphate solution (gradient elution from 58:42 to 75:25 in 18 minutes), using fluoromethasone as the internal substance. The detection wavelength was at 254 nm. The average recoveries of betamethasone sodium phosphate and betamethasone dipropionate were 101.3% (RSD, 1.3%, n = 9) and 99.0% (RSD = 1.3%, n = 9), respectively. The reproducibilities of the method (RSD) were 0.31% and 0.14% (n = 7), respectively.

De Wash et al. described the differentiation between dexamethasone and betamethasone in a mixture using multiple mass spectrometry [62]. Using GC-MS, the differentiation was based on a difference in the ratio of the ion traces of the two chromatographic peaks of the α and β epimer with m/z 310 and 330. A minimum
of 15% dexamethasone should be present in a mixture of both to detect it as present with a probability of 95%. In the same way betamethasone can be detected from 15% on. Because of the very similar structures of the dexamethasone and betamethasone epimers, no reversed-phase separations have been reported. Van Den Hauwe et al. reported simultaneous determination of betamethasone and dexamethasone residues in bovine liver by liquid chromatography/tandem mass spectrometry [63].

Lin and Wu reported the comparison between micellar electrokinetic chromatography and HPLC for the determination of betamethasone dipropionate, clotrimazole and their related substances [64]. The complete separation of a composite mixture that consisted of betamethasone dipropionate, clotrimazole and their derivatives in a pharmaceutical dosage form was achieved within 15 min using sodium dodecyl sulfate micellar electrokinetic chromatography. For the micellar electrokinetic chromatographic separations, electrophoretic media consisting of sodium dodecyl sulfate-phosphate buffer and various concentrations of alcohols or acetonitrile were used. The optimal condition for separating betamethasone dipropionate, clotrimazole and their analogues was found to be 50 mM sodium dodecyl sulfate-15% acetonitrile-5% butanol at pH 7.2.

Polettini et al. described different hyphenated liquid chromatographic (LC) and mass spectrometric (MS) techniques in order to set-up a method for the fast, direct analysis of betamethasone in hydrolysed and non-hydrolysed urine using large-volume sample injection [65]. After the optimisation of the LC parameters using a traditional UV detector and of the thermospray and mass spectrometric parameters by flow injection, urine samples (0.5 mL) were submitted to analysis by either LC combined with tandem mass spectrometry (MS-MS), coupled-column LC (LC-LC) combined with single quadrupole MS, and LC-LC-MS-MS.

Kedor-Hackmann et al. presented the determination of betamethasone dipropionate and salicylic acid in pharmaceutical preparations by high-performance liquid chromatography [66]. The method was standardized using a LiChrospher® 100 RP-18 (125 x 4 mm, 5 µm) column, acetonitrile-tetrahydrofuran-acetic acid 1% (25:20:55 v/v), apparent pH 3.3, as mobile phase, and UV detection at 254 nm. The peak area response versus concentration was
linear in a concentration range from 5.0 to 50.0 μg mL⁻¹ of betamethasone dipropionate and from 20.0 to 200.0 μg mL⁻¹ of salicylic acid. The correlation coefficients were 0.9997 for betamethasone dipropionate and 0.9987 for salicylic acid, and the relative standard errors of estimates were 1.38% for betamethasone dipropionate and 3.27% for salicylic acid. The coefficient of variation and the recovery average were, respectively, 0.411.15% and 100.09% for betamethasone dipropionate, and 0.57-0.95% and 99.79% for salicylic acid.

Amin and Issa reported a method for the spectrophotometric determination of betamethasone, based on the formation of charge-transfer complex with benzocaprol red and acid ethyl blue [67]. The calibration graph, resulting from the measurement of the absorbance of the chloroform and benzene extracts (10 mL) at 588 and 677 nm using benzocaprol red and acid ethyl blue respectively, was linear over the range 0-16.0 and 0-20.0 μg mL⁻¹ of betamethasone with relative standard deviation of 1.6 and 1.3% for 5 μg mL⁻¹ betamethasone using reagents and benzocaprol red and acid ethyl blue respectively. A simple and rapid densitometric method has been developed for simultaneous determination of betamethasone valerate and clioquinol in cream preparations [68]. After extraction of the analyte with acetone the extracts were spotted on silica gel plates which were eluted with ethyl acetate - n-butanol - 25% ammonia, 11 + 5 + 4 (v/v). Quantitative evaluation was performed by densitometric scanning of the analyte spots at λ = 247 nm in absorbance reflectance mode.

Chan et al. reported the structural determination of two isomers of betamethasone by NMR spectroscopy [69]. Two isomers of betamethasone 9α-fluoro-11β,17α,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione were isolated from the mother liquor of a manufacturing process and were characterized by NMR spectroscopy. They were identified as 8α-fluoro-11β,17α,21-trihydroxy-16β-methyl-9β-pregna-1,4-diene-3,20-dione (2) and 14β-fluoro-11β,17α,21-trihydroxy-16β-methyl-8α, 9β-pregna-1,4-diene-3,20-dione (3). The stereochemistries of 2 and 3 were assigned on the basis of proton-proton and proton-fluorine NOE experiments. The structure of the former was confirmed by x-ray crystallographic analysis.
Wu et al. presented the determination of betamethasone and dexamethasone in plasma by fluorogenic derivatization and liquid chromatography [70]. A simple and sensitive liquid chromatographic method was described for the determination of betamethasone and dexamethasone in plasma as fluorogenic derivatives. Betamethasone or dexamethasone in plasma after separation with a Sep-Pak C\textsubscript{18} cartridge was derivatized with 1-ethoxy-4-(dichloro-s-triazinyl)naphthalene. The resulting derivatives were separated on a reversed-phase C\textsubscript{18} column with acetonitrile-water (60:40, v/v) as the mobile phase. The linear range for the determination of each glucocorticoid in spiked plasma was over 2.5-50.0 pmol. The limit of detection for betamethasone or dexamethasone was 80.0 fmol per 20 µL injection (S/N = 5). The intraday relative standard deviations (n = 5) and the interday relative standard deviations (n = 3) were all less than 11% for both glucocorticoids. Recoveries for both glucocorticoids were greater than 90%.

Skrabalak and Henion described identification of betamethasone and a major metabolite in equine urine [71]. Skrabalak et al reported a method for the quantitative determination of betamethasone and its major metabolite in equine urine by micro-liquid chromatography-mass spectrometry [72]. Micro-liquid chromatography-mass spectrometry was utilized to quantitatively determine betamethasone and its major unconjugated metabolite, 6β-hydroxybetamethasone, in equine plasma and urine. The advantage of micro-liquid chromatography-mass spectrometry over conventional gas chromatography-mass spectrometry in corticosteroid determination was illustrated and the reliable, steadfast nature of micro-liquid chromatography-mass spectrometry was demonstrated through example.

A thin-layer chromatographic method suitable for the determination of the two epimeric corticosteroids dexamethasone and betamethasone in mixtures was described [73]. The procedure was based on the use of insoluble polyvinylpyrrolidone layers and anhydrous acid solvents. By means of the isonicotinic acid hydrazide fluorescence reagent, quantitation was possible by direct fluorimetry using multiple standard spots as reference points. By using this technique, up to 5% of each epimer in the presence of the other can be accurately determined. Downie et al. described the effect of betamethasone dipropionate
cream 0.05% (diprosone) on adrenocortical function [74]. This study was conducted with 16 subjects to determine whether the topical application of betamethasone dipropionate 0.05% cream, in amounts of 30 or 60 g daily for a period of 4 weeks, would lead to adrenocortical suppression. Five patients were given 60 g daily, 6 patients given 30 g daily and 5 normal subjects, 30 g daily. Adrenocortical function was assessed weekly by means of plasma cortisol levels. The results showed that transient adrenocortical suppression may follow the use of betamethasone dipropionate 0.05% cream daily at these dosage levels and this may a function of heightened absorption in some individuals.

The present work describes a new analytical method for the spectrophotometric determination of betamethasone using bromate-bromide and two dyes, crystal violet and xylene cyanol FF. The developed method has been successfully applied to the determination of betamethasone in pure and dosage form. Comparison of the proposed method with the earlier methods are shown in table 7.I.

7.1.3 APPARATUS
7.1.3.1 Spectrophotometer

A SHIMADZU (Model No: UV-2550) UV-Visible spectrophotometer with 1 cm matching quartz cells were used for the absorbance measurements.

7.1.4 REAGENTS AND SOLUTIONS

All reagents used were of analytical reagent grade and distilled water was used for the preparation of all solutions. A 1000 µgL⁻¹ standard drug solution of betamethasone (BM) was prepared in ethanol and the stock solution was diluted appropriately to get the working concentration. A bromate-bromide solution equivalent 1000 µg mL⁻¹ KBrO₃ and 10- fold excess of KBr was prepared by dissolving accurately weighed 100mg of KBrO₃ and 1g of KBr in water and diluting to the mark in a 100 mL calibrated flask. This was then diluted stepwise to obtain working concentrations of 10 µg mL⁻¹ KBrO₃ for use in betamethasone-crystal violet and betamethasone-xylene cyanol FF systems respectively. Hydrochloric acid (5M), crystal violet (CV) (0.05%), xylene cyanol FF (XFF) (0.05%) were also used.
7.1.5 PROCEDURES

7.1.5.1 Determination of Betamethasone Using Crystal Violet as a Reagent

Aliquots containing 2.00-8.00 µg mL$^{-1}$ of betamethasone were transferred into a series of 10 mL standard flasks using a micro burette. To this, 1mL of hydrochloric acid was added followed by 1.5 mL of bromate-bromide solution. Then 1mL of 0.05% of crystal violet was added. The contents were shaken well and diluted up to the mark with distilled water and mixed well. The absorbance of each solution was measured at 579 nm against the corresponding reagent blank.

7.1.5.2 Determination of Betamethasone Using Xylene Cyanol FF as a Reagent

Aliquots containing 0.80-2.40 µg mL$^{-1}$ of betamethasone were transferred into a series of 10 mL standard flasks using a micro burette. To this, 1mL of hydrochloric acid was added followed by 1.5 mL of bromate-bromide solution. Then 1mL of 0.05% of xylene cyanol FF was added. The contents were shaken well and diluted up to the mark with distilled water and mixed well. The absorbance of each solution was measured at 612 nm against the corresponding reagent blank.

7.1.5.3 Analysis of Dosage Forms

Betamethasone tablet was taken and the sample stock solution was prepared by grinding the tablet using a mortar and pestle and transferring to a 100 mL volumetric flask by washing with ethanol. The solution was shaken for 30 minutes and filtered through Whatman no.1 filter paper and the clear solution was made up to 100 mL with ethanol. Known amount of this solution was taken and analyzed for betamethasone content following the above procedure without any modification. The results are listed in table 7.I.1A and 7.I.1B.

7.1.6 RESULTS AND DISCUSSION

Spectrophotometric methods involve the addition of a measured excess of bromate-bromide in HCl medium and subsequent estimation of the residual bromine by reacting with a fixed amount of crystal violet or xylene cyanol FF followed by measurement of absorbance at 579 nm for betamethasone-crystal violet system and 612 nm for betamethasone-xylene cyanol FF system respectively. (fig. VII.A and VII.B and the reaction mechanism are shown in scheme 7.I.2A). Betamethasone, when added in increasing concentrations to a
fixed concentration of in situ generated bromine, consumes the latter proportionally and there occurs a concomitant fall in the concentration of bromine. When a fixed concentration of dye is added to decreasing concentrations of bromine, a concomitant increase in the concentration of dye results. In both the methods HCl medium is found to be ideally suited. It is found that maximum color developed within 20 minutes and remained almost stable for about 1 hour.

7. I 6.1 Analytical Data

Calibration graphs are linear over the concentration range 2.00–8.00 µg mL⁻¹ of betamethasone for betamethasone-crystal violet system and 0.80-2.40 µg mL⁻¹ of betamethasone for betamethasone-xylene cyanol FF system. The apparent molar absorptivity and Sandell’s sensitivity are calculated to be 0.52×10⁴ L mol⁻¹ cm⁻¹, 0.075 µg cm², 5.90×10⁴ L mol⁻¹ cm⁻¹, 0.006 µg cm² for betamethasone-crystal violet system and betamethasone-xylene cyanol FF system respectively. Slope, intercept and correlation coefficient for betamethasone-crystal violet system is found to be 0.012, 0.011, 0.9955, while that for betamethasone-xylene cyanol FF system is found to be 0.132, 0.019, 0.9889 respectively. The specificity test demonstrated that there is no interference in the determination of the drug. Adherence to Beer’s law for the determination of betamethasone using crystal violet and xylene cyanol FF are shown in fig. 7.I.A and 7.I.B.

7.I 6.2 Method Validation

To evaluate the accuracy and precision of the methods, pure drug within the working limits are analyzed, each determination being repeated five times. The results are shown in table 7.I.1C and 7.I.1D.

7.I 7 APPLICATIONS

The proposed method has been applied to the determination of betamethasone in pure and dosage forms. The percent recovery of added pure drug which lies between 99.0 and 101.2 reveals that the procedures are free from interference from usual tablet excipients like starch, glucose, calcium gluconate, sucrose, etc. Pure drug is analyzed within the working concentration limits to evaluate the accuracy and precision of the method. Each being repeated five times. The RSD (%) values are less than 2% which indicate the high accuracy and precision of the two methods (Table 7.I.1C and 7.I.1D). Statistical analysis of the
results by students t-test showed that the calculated t-value did not exceed the theoretical values for the two methods.

7.1.8 CONCLUSIONS

The results indicates that the UV spectrophotometric assay hold linearity, precision, accuracy, specificity and sensibility at concentration ranging from 2.00–8.00 \( \mu \text{g mL}^{-1} \) of betamethasone for betamethasone-crystal violet system and 0.80–2.40 \( \mu \text{g mL}^{-1} \) of betamethasone for betamethasone-xylene cyanol FF system, also the above results show the suitability of the proposed method for the spectrophotometric determination of betamethasone. The high selectivity and sensitivity make the method applicable for routine analysis of betamethasone from tablet formulation.
**TABLE 7.1: COMPARISON OF THE PROPOSED METHOD WITH THE EARLIER METHOD.**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Range</th>
<th>λ max (nm)</th>
<th>Molar absorptivity (L mol⁻¹ cm⁻¹)</th>
<th>Method</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzocaprol red</td>
<td>0-16.00</td>
<td>588</td>
<td>1.55×10⁴</td>
<td>Spectrophotometry</td>
<td>[67]</td>
</tr>
<tr>
<td>Acid ethyl blue</td>
<td>0-20.00</td>
<td>677</td>
<td>1.43×10⁴</td>
<td>Spectrophotometry</td>
<td>[67]</td>
</tr>
<tr>
<td><strong>Proposed method</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Using crystal violet</td>
<td>2.00-8.00</td>
<td>579</td>
<td>0.52×10⁴</td>
<td>Spectrophotometry</td>
<td></td>
</tr>
<tr>
<td>Using xylene cyanol FF</td>
<td>0.80-2.40</td>
<td>612</td>
<td>5.90 ×10⁴</td>
<td>Spectrophotometry</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 7.1A: RESULTS OF ASSAY OF FORMULATIONS BY THE PROPOSED METHOD USING CV AS A REAGENT.**

<table>
<thead>
<tr>
<th>Sample</th>
<th>BM certified (mg)</th>
<th>Found± SD¹</th>
<th>Recovery (%)</th>
<th>²t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Betnesol</td>
<td>0.50</td>
<td>0.496±0.01</td>
<td>99.20</td>
<td>0.813</td>
</tr>
</tbody>
</table>

¹Mean±Standard deviation (n=5) [mg/tablet]; ²Tabulated t-value at 95% confidence level is 2.31; Betamethasone tablet- GlaxoSmithKline pharmaceuticals Limited

**TABLE 7.1B: RESULTS OF ASSAY OF FORMULATIONS BY THE PROPOSED METHOD USING XFF AS A REAGENT.**

<table>
<thead>
<tr>
<th>Sample</th>
<th>BM certified (mg)</th>
<th>Found± SD¹</th>
<th>Recovery (%)</th>
<th>²t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Betnesol</td>
<td>0.50</td>
<td>0.498±0.01</td>
<td>99.60</td>
<td>0.372</td>
</tr>
</tbody>
</table>

¹Mean±Standard deviation (n=5) [mg/tablet]; ²Tabulated t-value at 95% confidence level is 2.31; Betamethasone tablet-GlaxoSmithKline pharmaceuticals Limited
**TABLE 7.1.C: EVALUATION OF ACCURACY AND PRECISION**  
**BETAMETHASONE (USING CV AS A REAGENT)**

<table>
<thead>
<tr>
<th>Amount taken (µg mL⁻¹)</th>
<th>Amount founda (µg mL⁻¹)</th>
<th>Recovery (%)</th>
<th>SD</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.00</td>
<td>1.98</td>
<td>99.00</td>
<td>0.02</td>
<td>1.01</td>
</tr>
<tr>
<td>3.00</td>
<td>3.01</td>
<td>100.30</td>
<td>0.02</td>
<td>0.66</td>
</tr>
<tr>
<td>4.00</td>
<td>3.99</td>
<td>99.80</td>
<td>0.02</td>
<td>0.50</td>
</tr>
<tr>
<td>5.00</td>
<td>5.02</td>
<td>100.40</td>
<td>0.05</td>
<td>0.99</td>
</tr>
<tr>
<td>6.00</td>
<td>6.01</td>
<td>100.20</td>
<td>0.04</td>
<td>0.67</td>
</tr>
</tbody>
</table>

*a-Average of five determinations, SD- Standard deviation

**TABLE 7.1.D- EVALUATION OF ACCURACY AND PRECISION**  
**BETAMETHASONE (USING XFF AS A REAGENT)**

<table>
<thead>
<tr>
<th>Amount taken (µg mL⁻¹)</th>
<th>Amount founda (µg mL⁻¹)</th>
<th>Recovery (%)</th>
<th>SD</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.80</td>
<td>0.79</td>
<td>99.75</td>
<td>0.01</td>
<td>1.25</td>
</tr>
<tr>
<td>1.00</td>
<td>1.01</td>
<td>101.00</td>
<td>0.02</td>
<td>1.98</td>
</tr>
<tr>
<td>1.20</td>
<td>1.20</td>
<td>100.16</td>
<td>0.01</td>
<td>0.83</td>
</tr>
<tr>
<td>1.40</td>
<td>1.40</td>
<td>100.14</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>1.60</td>
<td>1.60</td>
<td>100.12</td>
<td>0.03</td>
<td>0.02</td>
</tr>
<tr>
<td>1.80</td>
<td>1.82</td>
<td>101.20</td>
<td>0.008</td>
<td>0.44</td>
</tr>
</tbody>
</table>

*a-Average of five determinations, SD- Standard deviation
**FIGURE VII. I.1:** ABSORPTION SPECTRUM FOR BETAMETHASONE CV SYSTEM

![Absorption Spectrum for Betamethasone CV System](image1)

**FIGURE VII. I.2:** ABSORPTION SPECTRUM FOR BETAMETHASONE-XFF SYSTEM

![Absorption Spectrum for Betamethasone-XFF System](image2)
FIGURE 7.I.A: ADHERENCE TO BEER’S LAW FOR THE DETERMINATION OF BETAMETHASONE USING CVAS A REAGENT

![Image of Figure 7.I.A](image)

FIGURE 7.I.B: ADHERENCE TO BEER’S LAW FOR THE DETERMINATION OF BETAMETHASONE USING XFF AS A REAGENT

![Image of Figure 7.I.B](image)
REACTION SCHEME 7.I.2A

Known excess bromate -bromide

\[ \text{Method 1} \]

Crystal Violet

Bleached Crystal Violet, measured at 579 nm

\[ \text{Method 2} \]

Xylene cyanol FF

Bleached Xylene cyanol FF, measured at 612 nm
222


