Chapter 4

Literature Survey

A thorough literature survey has revealed that most of the selected drugs may or may not be official in Pharmacopoeia and a number of analytical methods are available for their individual estimation or in combination with some other drug but no analytical method is reported for the simultaneous estimation of the drugs in standard laboratory mixture or in commercial formulation available in the Indian market. Therefore some selective methods reported for the drugs selected present study are mentioned in the following section

4.1 Butenafine hydrochloride[1,2]

4.1.1 Simultaneous HPLC Determination of Butenafine Hydrochloride and Betamethasone in a Cream Formulation

A fast, specific, accurate and precise reverse phase high performance liquid chromatographic method was developed for the simultaneous determination of butenafine hydrochloride and betamethasone in cream formulation. The determination was carried out on licrocart licrosphere RP-select B (250×4.6 mm, 5 µ) column in isocratic mode, the mobile phase consisting of 50 mM ammonium acetate buffer and acetonitrile in the ratio of 60:40, adjusted to pH 4.5 ± 0.1 with glacial acetic acid. The flow rate was 2.0 ml/min and eluent was monitored at 254 nm. The retention times of butenafine hydrochloride and betamethasone were 4.70 min and 7.76 min, respectively, and the resolution factor was greater than 4.0. Linearity of butenafine hydrochloride and betamethasone were in the range of 100-300 µg/ml and 5-15 µg/ml, respectively. The proposed method is also found to be precise and robust for the simultaneous determination of butenafine hydrochloride and betamethasone in cream formulation.

4.1.2 Stability-Indicating LC Assay for Butenafine Hydrochloride in Creams Using an Experimental Design for Robustness Evaluation and Photodegradation Kinetics Study

A stability-indicating liquid chromatography method for the determination of the antifungal agent butenafine hydrochloride (BTF) in a cream was developed and validated using the Plackett-Burman experimental design for robustness evaluation. Also, the drug photodegradation kinetics was determined. The analytical column was operated with acetonitrile, methanol and a solution of triethylamine 0.3% adjusted to pH 4.0 (6:3:1) at a flow rate of 1 mL/min and detection at 283 nm. BTF extraction from the cream was done with *n*-butyl alcohol and methanol in ultrasonic bath. The performed degradation conditions were: acid and basic media with HCl 1M and NaOH 1M, respectively, oxidation with H_2O_2 10%, and the exposure to UV-C light. No

interference in the BTF elution was verified. Linearity was assessed (r^2 = 0.9999) and ANOVA showed non-significative linearity deviation ($p > 0.05$). Adequate results were obtained for repeatability, intra-day precision, and accuracy. Critical factors were selected to examine the method robustness with the two-level Plackett-Burman experimental design and no significant factors were detected ($p > 0.05$). The BTF photodegradation kinetics was determined for the standard and for the cream, both in methanolic solution, under UV light at 254 nm. The degradation process can be described by first-order kinetics in both cases.

4.2 Betamethasone Dipropionate [3, 4, 5]

4.2.1 Densitometric determination of betamethasone dipropionate and salicylic acid in lotions, and validation of the method

A simple and rapid densitometric method has been developed for determination of betamethasone dipropionate and salicylic acid in lotions. 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 + 20 + 14 + 0.5 (*v/v*). Quantitative evaluation was performed by measuring the absorbance reflectance of the betamethasone dipropionate and salicylic acid spots at λ = 250 and 310 nm, respectively. This densitometric TLC method is selective, precise, and accurate and can be used for routine analysis of lotions in pharmaceutical industry quality-control laboratories.

4.2.2 Development and validation of a stability-indicating HPLC method for simultaneous determination of salicylic acid, betamethasone dipropionate and their related compounds in Diprosalic Lotion.

Diprosalic Lotion is an anti-inflammatory drug product that contains salicylic acid and betamethasone dipropionate as active pharmaceutical ingredients (APIs). A reversed-phase high performance liquid chromatography (RP-HPLC) method was developed for simultaneous determination of salicylic acid, betamethasone dipropionate, and their related compounds in Diprosalic Lotion. A 150 mm x 4.6 mm I.D. YMC J'sphere ODS-H80 column at 35 degrees C and UV detection at 240 nm was used. A gradient elution was employed using 0.05% (v/v) methanesulfonic acid solution and acetonitrile as mobile phases. A total of thirty three compounds from Diprosalic Lotion samples were separated in 38 min. The stability-indicating capability of this method has been demonstrated by the adequate separation of all the impurities

and degradation products in expired stability samples of Diprosalic Lotion. The method was validated as per the current ICH guidelines.

4.2.3 Development and validation of a stability-indicating RP-HPLC method for simultaneous assay of betamethasone dipropionate, chlorocresol, and for the estimation of betamethasone dipropionate related compounds in a pharmaceutical cream and ointment.

A new stability-indicating reversed-phase HPLC (RP-HPLC) method has been developed and validated for simultaneous assay of betamethasone dipropionate (BD) and chlorocresol and also for the estimation of BD related compounds in a pharmaceutical cream matrix. In addition, this newly developed RP-HPLC method was also demonstrated as suitable for a pharmaceutical ointment product that does not contain chlorocresol. The RP-HPLC method uses a Waters SymmetryShield RP18 analytical column (150 4.6 mm). Water (mobile phase A) and acetonitrile (mobile phase B) were used in the gradient elution with a flow rate of 1.5 mL/min and detection wavelength at 240 nm. A Waters XBridge Shield RP18 analytical column (150 4.6 mm) was identified as an alternate column. The limit of detection (LOD) and the limit of quantitation (LOQ) are 0.02 g/mL and 0.05 g/mL, respectively. The precision of the method for BD is less than 0.3% RSD, and the accuracy of BD ranged from 99.5% to 102.6%. The stability-indicating capability of this method has been demonstrated by analyzing aged samples of the product. This RP-HPLC method was successfully validated per ICH guidelines and proved to be suitable for routine quality control use.

4.3 Escitalopram oxalate[6-14]

4.3.1 Spectrophotometric and reversed-phase high-performance liquid chromatographic methods for simultaneous determination of Escitalopram oxalate and Clonazepam in combined tablet dosage form

Simple, accurate, precise, and sensitive ultraviolet spectrophotometric and reversed-phase high-performance liquid chromatographic (RP-HPLC) methods for simultaneous estimation of Escitalopram oxalate (ESC) and Clonazepam (CLO) in combined tablet dosage form have been developed and validated. The spectroscopic method employs an absorbance correction method using 238.6 and 308 nm as 2 wavelengths for estimation with methanol and water as solvents. Beer's law is obeyed in the concentration range of 10.0-50.0 and 0.5-3.0 micro/mL for ESC and CLO, respectively. The RP-HPLC method uses a Jasco HPLC system with HiQ SiL C18 column

(250 x 4.6 mm id) acetonitrile-0.005 M tetrabutylammonium hydrogen sulfate (55 + 45, v/v) as the mobile phase, and satranidazole as an internal standard. The detection was carried out using an ultraviolet detector set at 287 nm. For the HPLC method, Beer's law is obeyed in the concentration range of 10.0-60.0 and 0.5-3.0 microg/mL for ESC and CLO, respectively. Both methods have been successfully applied for the analysis of the drugs in a pharmaceutical formulation. Results of analysis were validated statistically and by recovery studies.

4.3.2 Simultaneous HPTLC Determination of Escitalopram Oxalate and Clonazepam in Combined Tablets .

A new, simple high-performance thin-layer chromatographic method has been established and validated for simultaneous determination of Escitalopram oxalate and Clonazepam in a combined tablet dosage form. The drugs were separated on aluminum plates precoated with silica gel 60 F_{254}; toluene–ethyl acetate–triethylamine 7:3.5:3 (*v/v*) was used as mobile phase. Quantitative analysis was performed by densitometric scanning at 258 nm. The method was validated for linearity, accuracy, precision, and robustness. The calibration plot was linear over the ranges 250–2,500 and 50–500 ng band^{-1} for Escitalopram oxalate and Clonazepam, respectively. The method was successfully applied to the analysis of drugs in a pharmaceutical formulation.

4.3.3 Development and validation of liquid chromatographic method For estimation of Escitalopram oxalate in tablet dosage forms.

A simple, specific, accurate and precise RP-HPLC method was developed and validated for the determination of Escitalopram oxalate in tablet dosage forms. A hypersil BDS C8, 5- column having 250x4.6mm internal diameter in isocratic mode with mobile phase containing methanol: disodium hydrogen phosphate: acetonitrile (28:44:28v/v, pH 7.0±0.05) was used. The flow rate was 1.5ml/min and effluents were monitored at 226nm. The retention time of Escitalopram oxalate was 8.45 min. The linearity range is 250-1500-g/ml with coefficient of correlation 0.9999. The method was validated in terms of accuracy, precision, repeatability. The percentage recovery for Escitalopram oxalate was found to be 99.0%. The proposed method was successfully applied for quantitative determination of Escitalopram oxalate in single dosage form for routine analysis.

4.3.4 Colorimetric method for the estimation of Escitalopram oxalate in tablet dosage form

A colorimetric method for the analysis of Escitalopram oxalate in pure form and in tablets has been developed based on the formation of chloroform soluble ion associates with bromocresol green acidic dye. The extract of ion associates exhibited absorption maxima at 417 nm obeying Beer«SQ»s law in the range of 2-10 µg/ml. The method is simple, precise and accurate with recovery of 98-102% and does not require any separation of

4.3.5 Zero order spectrophotometric method for estimation of Escitalopram oxalate in tablet formulations

A new, simple, fast and reliable zero order spectrophotometric method has been developed for determination of Escitalopram Oxalate in bulk and tablet dosage forms. The quantitative determination of drug was carried out using the zero order values (absorbance) measured at 238 nm. Calibration graph constructed at 238 nm was linear in concentration range of 2-20 µg/ml with correlation coefficient 0.9999. The method was found to be precise, accurate, specific, and validated as per ICH guidelines and can be used for determination of Escitalopram Oxalate in tablet formulations.

4.3.6 Spectrophotometric Method for Simultaneous Estimation of Escitalopram Oxalate and Clonazepam in Tablet Dosage Form

A simple, accurate and precise spectrophotometric method has been developed for simultaneous estimation of Escitalopram oxalate and Clonazepam in combined dosage form. Simultaneous equation method is employed for simultaneous determination of Escitalopram oxalate and Clonazepam from combined dosage forms. In this method, the absorbance was measured at 238 nm for Escitalopram oxalate and 273 nm for Clonazepam. Linearity was observed in range of 5-100 µg/ml and 5-50 µg/ml for Escitalopram and Clonazepam respectively. Recovery studies confirmed the accuracy of proposed method and results were validated as per ICH guidelines. The method can be used for routine quality control of pharmaceutical formulation containing Escitalopram and Clonazepam.

4.3.7 Study on Pharmacokinetics of Escitalopram Oxalate Tablets in Human Body

Es-citalopram oxalate tablets were administered orally at a single dose of30mg to10healthy subjects respectively,the plasma concentration of Escitalopram oxalate was determined by HPLC method,the pharmacokinetic parameter was fitted with3p97software.

RESULTS: The concentration-time curve of Escitalopram oxalate tablets was in line with the two-compartment model,the main pharmacokinetics parameters of Escitalopram oxalate were as follows:

Cmax was (42.73±10.19) μg·L, tmax was (2.90±0.32) h, t1/2 was (35.34±7.78) h, AUC0~132 was (1241.5±194.3) (μg·h)/L and the AUC0~∞ was (1327.5±210.5) (μg·h)/L.

CONCLUSION: The study on pharmacokinetics can be used as a reference in the clinical medication.

4.3.8 Pharmacokinetics of Escitalopram in healthy volunteers

The volunteers were given a single oral dose of 5,10 or 20 mg Escitalopram for single dose pharmacokinetics, or 10 mg Escitalopram tablets once a day for 10-days multidose and steady-state study. The plasma concentrations of Escitalopram were determined by a validated HPLC-MS/MS method. The pharmacokinetic parameters were calculated by DAS 2.0 software.

RESULTS: The main pharmacokinetic parameters of Escitalopram after the single oral dose of 5, 10 or 20 mg were as follows:

AUC(0-144) were (219±s 49), (367±60) and (689±174) (μg·h)/L, respectively;AUC(0-∞) (242±64), (414±79) and (745±207) (μg·h)/L; Cmax (5.5±1.0), (8.8±1.3) and (21±6)μg/L; tmax (4±3), (6±3) and (3.0±1.5) h; t1/2 (40±11), (48±10) and (37±6) h; MRT (56±13), (63±11) and (51±7) h.

The parameters after multidose of 10 mg were as follows:

AUC(0-144) was (914±202)μg·h/L, AUC(0-∞) (993±214)μg·h/L, AUC(ss) (364±78)μg·h/L, Cmax~(ss) (26±4)μg·h/L, Cmin~(ss) (12.4±1.1) μg·h/L, Cav~(ss) (15±3) μg·h/L, DF 0.87±0.22, tmax (2.6±1.7)h, t1/2 (40±7) h.

4.3.9 Method for the estimation of Escitalopram in bulk and in dosage Forms

The present study indicates a simple, accurate and precise RP- HPLC method for the estimation of Escitalopram in bulk and in pharmaceutical formulations. The mobile phase used was phosphate buffer with pH 7.0 and an organic mixture solvent (acetonitrile and methanol in the ratio of 1:1 v/v). Then the mobile phase was prepared by mixing buffer solution and mixture of organic solvents in the ratio of (55: 45 v/v) respectively. The specification of the chromatographic system 150 mm ×4.6 mm Xterra RP 18, 5 μm, flow rate 1.2ml/min, detection 238nm, injection volume 10μl and run time 10 min. Only very few HPLC procedures have been

reported in the literature for the determination of Escitalopram in pharmaceutical formulations and biological fluids. There are no reports for the determination of Escitalopram by HPLC in pure form. Hence I have made an attempt to develop a HPLC method for the determination of Escitalopram in bulk and in pharmaceutical formulations.

4.4 Etizolam[15-18]

4.4.1 Simple method for the determination of benzodiazepines in human body fluids by high-performance liquid chromatography–mass spectrometry

4.4.2 Rapid and sensitive detection of benzodiazepines and zopiclone in serum using high-performance thin-layer chromatography

4.4.3 Effects of different cyclodextrins on the morphology, loading and release properties of poly (DL-lactide-co-glycolide)-microparticles containing the hypnotic agent etizolam

4.4.4 Simultaneous determination of etizolam, triazolam and their metabolites by gas chromatography tandem mass spectrometry.

4.5 Albendazole[19-23]

4.5.1 Developing a Spectrophotometric method for the estimation of Albendazole in solid and suspension forms

A Spectrophotometric method has been developed for the determination of Albendazole in bulk, tablet and suspension dosage forms. Solution of Albendazole in methanolic glacial acetic acid solution shows maximum absorbance at 235 nm. Beer's law was obeyed in the concentration range of 2.5 - 20 _g/ml with molar absorptivity of 1.0815*104 mol^{-1} cm^{-1}. The method was applied for the analysis of the drug in the pure, tablet and suspension forms. The slope and intercept of the equation of the regression line are 0.0310 and 0.00067 respectively. Correlation coefficient was found to be 0.9998. Results of percentage recovery showed that the method was not affected by the presence of common excipients. The proposed method is simple, sensitive, rapid, economical and could find application as an inprocess quality control method for Albendazole.

4.5.2 RP-HPLC Method for Simultaneous Estimation of Levamisole, Mebendazole and Albendazole in Pharmaceutical Products

Single and reproducible RP-HPLC method has been developed for the simultaneous estimation of Levamisole, Mebendazole and Albendazole in pharmaceutical products. Chromatographic

separation was achieved by using Inertsil ODS-3V C18, 250 x 4.6 mm, 5µm column, mobile phase composed of sol-A: Potassium dihydrogen phosphate (1.0 gram in 1000 ml of HPLC Water) buffer and sol-B: Acetonitrile with gradient elution (0-5min- sol-A: 80-80; 5-7min- sol-A: 80-60; 7- 10min- sol-A: 60-30; 10-15min- sol-A: 30-80 and 15-20min- sol-A: 80-80). Flow rate was 1.00 ml per min and measured the absorbance at 210nm. The retention time of Levamisole, Mebendazole and Albendazole are 4.8min, 12.8min and 14.1min, respectively. The linearity of the method was evaluated from 5µg per mL to 100µg per mL for each ingredient and the correlation coefficient result was observed for each ingredient was not less than 0.999. The developed method has wide applicable in the quantification of Levamisole, Mebendazole and Albendazole in pharmaceutical dosage forms.

4.5.3 Quantification of Albendazole in Dewormer Formulations in the Kenyan market

In this study, the amount of active ingredient, Methyl [5-(propylthio)-2-benzimidazolecarbamate] (albendazole) in dewormer formulations was quantified using High Performance Liquid Chromatography (HPLC) and Ultra–Violet/ Visible (UV/ Vis) Spectrophotometer. Dewormer samples were obtained from various drug stores in Nairobi city.The analyses results indicated that in a number of cases the concentrations of albendazole differed with that indicated on the manufacturers' labels. In two cases the concentration of albendazole grossly differed from other samples.

4.5.4 Validated UV Spectrophotometric Method for estimation of Albendazole in Tablet

Albendazole (ABZ) is an oral broad-spectrum anthelmintic, antiparasitic agent generally prescribed for the treatment of tissue infections caused by a variety of nematodes. No UV method is reported for routine analysis of albendazole. Here we have developed simple, accurate and rapid UV spectrophotometric method for estimation of albendazole from Tablet formulation. The drug obeyed the Beer's law and showed good correlation. It showed absorption maxima at 298 nm in Dimethyl Formamide (DMF). The linearity was observed between 2-20 µg/ml. The results of analysis were validated by recovery studies. The recovery was found to be 99.43-101.55%. The method was found to be simple, reliable, rapid, precise, specific and reproducible and can be applied for routine analysis of albendazole in different dosage form and dissolution studies.

4.5.5 A Bioequivalence Study of an Albendazole Oral Suspension Produced in Iran and a Reference Product in Sheep

In a parallel design, a single oral dose ofalbendazole (ABZ), 5 mg/kg, was administered to 2 groups of 8 sheep to study the bioequivalence of an ABZ oral suspension produced in an Iranian pharmaceutical company and a reference product (Valbazen®, Pfizer Inc.). A third group of 8 sheep without dosing was used as control. Blood samplesof all groups were collected at specified times within 0-72 hours post-dosing. The serum levels of albendazole sulphoxide (ABZ-SO), the main metabolite of ABZ, were determined using a high performanceliquid chromatographic method with ultraviolet detection. Peak areas were used for calculatingABZ-SO concentrations and ABZ-SO pharmacokinetic parameters obtained using non-compartmental analysis. Statistical analysis of data pointed out that there were significant differences between the area under the concentration-time curve and peak serum concentration of these products, although there was no significant differencein their time to peak serum. Albendazole is poorly absorbed from the GI tract. This property, which is ideal for its use against luminal parasitic infections, is a problem in the treatment of systemic diseases. After oral administration of ABZ, it is metabolized rapidly to a pharmacologically active metabolite, albendazole sulfoxide (ABZ-SO), and it constitutes the main part of drug in blood.1,4,5 Because the first-pass metabolism for ABZ is extensive and ABZ serum level is negligible after its oral dosing in sheep,1,5 the kinetic profile of ABZ-SO was used for comparison of bioavailability of 2 oral ABZ products in the present study.

4.6 Ivermectin[24-26]

4.6.1 Liquid chromatographic assay of Ivermectin in human plasma for application to clinical pharmacokinetic studies

There is a need for an accurate, sensitive and selective high-performance liquid chromatography (HPLC) method for the quantitation of Ivermectin in human plasma that separates the parent drug from metabolites. Ivermectin and the internal standard, Moxidectin, were extracted from 0.2 ml of human plasma using Oasis HLB solid phase extraction cartridges. After extraction, fluorescent derivatives of Ivermectin and Moxidectin were made by reaction with trifluoroacetic anhydride and *N*-methylimidazole. Separation was achieved on a Alltech Ultrasphere C18 5µ column with a mobile phase composed of tetrahydrofuran:acetonitrile:water (40:38:22 v/v/v). Detection is by fluorescence, with an excitation of 365 nm and emission of 475 nm. The

retention times of Ivermectin and internal standard, Moxidectin are approximately 24.5 and 12.5 min, respectively. The assay is linear over the concentration range of 0.2–200 ng/ml of Ivermectin in human plasma (r = 0.9992, weighted by 1/concentration). Recoveries of Ivermectin are greater than 80% at all concentrations. The analysis of quality control samples for Ivermectin 0.2, 25, and 200 ng/ml demonstrated excellent precision with coefficient of variation of 6.1, 3.6 and 2.3%, respectively (n = 6). The method is accurate with all intra-day (n = 6) and interday (n = 12) mean concentration within 10% of nominal values at all quality control sample concentrations. Storage stability for 30 days at −80 °C and after three freeze–thaw cycles are within acceptable limits. The method separates Ivermectin from multiple less and more polar unidentified metabolites. This method is robust and suitable for clinical pharmacokinetic studies. The analytical procedure has been applied to a pharmacokinetic study of Ivermectin in healthy volunteers and to the analysis of plasma specimens from patients with disseminated strongyloidiasis.

4.6.2 Simultaneous Analysis of Ivermectin and Clorsulon in Injection Solutions by High Performance Liquid Chromatography with Confirmation by Liquid Chromatography Mass Spectrometry

Ivermectin and Clorsulon are antiparasitic agents used in veterinary medicine, sometimes used in combination. The purpose of this work was to develop a method for the simultaneous analysis of Ivermectin and Clorsulon in injection solutions by high-performance liquid chromatography (HPLC), to differentiate between two Ivermectin analogs, and to confirm structures by ultraviolet (UV) spectroscopy and mass spectrometry.

Methods: Ivermectin and Clorsulon were analyzed by HPLC with detection by UV at 244 nm and by negative ion atmospheric pressure chemical ionization liquid chromatography mass spectrometry (APCI LC-MS). Due to a significant difference in the polarity of Ivermectin and Clorsulon, a mobile solvent gradient and a short (4.6 mm x 50 mm) C-18 column were used to provide separation with a reasonable analysis time.

4.6.3 Determination of Ivermectin stability by high-performance thin-layer Chromatography

A rapid, sensitive and stability signifying high-performance thin-layer chromatographic (HPTLC) method was developed and validated for the quantitative estimation of Ivermectin

(IVM) as a bulk drug and in pharmaceutical formulations. The separation was achieved on Lichrospher TLC aluminum plates pre-coated with silica gel 60F-254 (20cm×10cm×200cm) using *n*-hexane:acetone:ethylacetate (6.5:3.5:0.1 v/v/v) as mobile phase. The densitometric analysis was carried out at 247 nm wavelength.

Compact spots of IVM were found at *R*f = 26.02. For proposed procedure, linearity ($r2$ = 0.9989), limit of quantification (24.9 ng $spot^{-1}$), limit of detection (8.22 ng $spot^{-1}$) recovery (98.25–100.16%), and inter as well intra-day precision (≤2.21) was found to be satisfactory. We have synthesized polymeric nanoparticles encapsulated formulation of Ivermectin (IVM-NPs); utilizing micel large aggregates of crosslinked random copolymer Nisopropylacrylamide (NIPAAM) with N-vinyl-2-pyrrolidone (VP) and polyethyleneglycol monoacrylate (PEG-A) for lymphatic targeting and it was also quantified by the developed method. IVM and formulations were subjected to acid and alkali hydrolysis, oxidation and photo-degradation. The drug undergoes degradation under acidic, basic, light and oxidation conditions. This indicates that the drug is susceptible to acid- base hydrolysis, oxidation and photo-oxidation and the developed method is selective for quantifying IVM even in the presence of degradatnts. The method was applicable for routine analysis and stability testing of IVM in pharmaceutical drug delivery systems. As the method could effectively separate the said drug from its degradation products, it can be employed as a stability indicating one

4.7 Racecadotril [27-30]

4.7.1 New spectrophotometric methods for the determination of Racecadotril in bulk drug and capsules

Two simple and sensitive spectrophotometric methods (A and B) for the determination of Racecadotril in bulk drugs and pharmaceutical formulations are described. In method A, methanol was used as solvent and shows absorption maximum at 231 nm. In method B, the solvent used was acetonitrile:water in the ratio of 1:3 and shows absorption maximum at 232 nm. The Beer's law range for method A is 25-100 mg/ml and 20-80 mg/ml for method B. When capsules dosage forms were analyzed, the results obtained by the proposed methods are in good agreement with the labeled amounts and the results were validated statistically.

There is a need for an accurate, sensitive and selective high-performance liquid chromatography (HPLC) method for the quantitation of Ivermectin in human plasma that separates the parent

drug from metabolites. Ivermectin and the internal standard, Moxidectin, were extracted from 0.2 ml of human plasma using Oasis HLB solid phase extraction cartridges. After extraction, fluorescent derivatives of Ivermectin and Moxidectin were made by reaction with trifluoroacetic anhydride and *N*-methyl-imidazole. Separation was achieved on a Alltech Ultrasphere C18 5μ column with a mobile phase composed of tetrahydrofuran:acetonitrile:water (40:38:22 v/v/v). Detection is by fluorescence, with an excitation of 365 nm and emission of 475 nm. The retention times of Ivermectin and internal standard, Moxidectin are approximately 24.5 and 12.5 min, respectively. The assay is linear over the concentration range of 0.2–200 ng/ml of Ivermectin in human plasma (r = 0.9992, weighted by 1/concentration). Recoveries of Ivermectin are greater than 80% at all concentrations. The analysis of quality control samples for Ivermectin 0.2, 25, and 200 ng/ml demonstrated excellent precision with coefficient of variation of 6.1, 3.6 and 2.3%, respectively (n = 6). The method is accurate with all intra-day (n = 6) and interday (n = 12) mean concentration within 10% of nominal values at all quality control sample concentrations. Storage stability for 30 days at −80 °C and after three freeze–thaw cycles are within acceptable limits. The method separates Ivermectin from multiple less and more polar unidentified metabolites. This method is robust and suitable for clinical pharmacokinetic studies. The analytical procedure has been applied to a pharmacokinetic study of Ivermectin in healthy volunteers and to the analysis of plasma specimens from patients with disseminated strongyloidiasis. A simple, precise and rapid RP-HPLC method was developed for the determination of Racecadotril in a pharmaceutical formulation using Gemfibrozil as internal standard. Ratio of the peak area of analyte to internal standard was used for quantification. The chromatographic separation was carried out by using a Reverse Phase C18 column (BDS-Hypersil). The mobile phase consisting of a mixture of 20 mM phosphate buffer (pH 3.5) and acetonitrile in the ratio of (40:60) with detection at 230 nm at a flow rate of 1 ml/min was employed. The method was statistically validated for linearity, accuracy and precision. The elution time was 6.9 min for Racecadotril and 9.8 min for Gemfibrozil. The simplicity and accuracy of the proposed method ensures its use in routine quality control analysis of pharmaceutical formulations.

4.7.2 Development and validation of a rapid RP-HPLC method for the determination of Racecadotril in formulation

A simple rapid specific precise and accurate reverse phase high performance liquid chromatographic method was developed for the determination of Racecadotril (RACE) in sachet dosage forms using Atorvastatin as internal standard. Ratio of the peak area of analyte to internal standard was used to calculate for quantification. A phenomenex-Luna RP-18, 5mm column having 250x4.6 mm i.d. in isocratic mode, with mobile phase containing acetonitrile: 0.05M phosphate buffer (potassium dihydrogen orthophosphate): triethylemine (80:19.95:0.05) adjusted to pH 3.95 using orthophosphoric acid. The flow rate was 1.0ml/min and effluents were monitored at 231nm. The retention times of Atorastatin calcium and Racecadotril were 3.453 min and 4.210 min respectively. Linearity was observed over concentration range of 10-80 mg/ml. The recovery of Racecadotril was found to be in the range of 99.6-100.5%. The proposed method was validated successfully and applied to the estimation of a Racecadotril in sachet dosage forms.

4.7.3 Spectrophotometric and spectrofluorimetric methods for determination of Racecadotril

Two accurate and sensitive spectrophotometric and spectrofluorimetric methods were developed for determination of Racecadotril. In the first method reduction of Fe3+ into Fe2+ in presence of o-phenanthroline by Racecadotril to form a stable orange-red ferroin chelate [Fe- (Phen)3]2+ was the basis for its determination . The absorbance at 510 nm was measured and linear correlation was obtained in the concentration range of 2.5 – 25μg mL^{-1}. In the second method the native fluorescence of Racecadotril in acetonitrile solvent at $\lambda = 319$ nm when excitation was at 252 nm is used for its determination. Linear correlation was obtained in the concentration range of 50 to 500 ng mL-1. The proposed methods were applied for determination of Racecadotril in bulk powder with mean accuracy of 100.39±1.239 for the spectrophotometric method and 100.09± 1.042 for the spectrofluorimetric method. The proposed methods were successfully applied for determination of Racecadotril in its pharmaceutical dosage form.

4.7.4 Determination of Racecadotril and its impurities by HPLC

A HPLC method was established for the determination of Racecadotril and its impurities.METHODS A ODS column was used and the mixture of acetonitrile□ potassium

dihydrogen phosphate (KH_2PO_4)solution (70: 30) was used as the mobile phase.The detection wavelength was 210 nm.

RESULTS: The liner range of the Racecadotril was 0.08～0.24 $mg \cdot mL^{-1}$ and the regression equation was y=15847x+3873 (r^2=0.9997). The liner range of benzylthiorphan disulphide was 2.40～21.56$\mu g \cdot mL^{-1}$ and the regression equation was Y=1826x+46 (r^2=0.9999). The measurable lowest limit was $1\mu g \cdot mL^{-1}$.The average recovery was 100.0%.

CONCLUSION: The method was convenient, accurate and specific.

4.8 Fluocinolone acetonide [31-33]

4.8.1 Development of a reversed-phase HPLC method for analysis of fluocinolone acetonide in gel and ointment

Fluocinolone acetonide and additives in gel. Drugs were chromatographed on a C_{18} reversed-phase column with 55:45 (v/v) methanol–water as mobile phase and detection at 238 nm. Solution concentrations were measured on a weight basis to avoid the use of an internal standard. The method was statistically validated for linearity, accuracy, precision, and selectivity. Linearity for assay of fluocinolone acetonide, methyl 4-hydroxybenzoate (nipagin M), and propyl 4-hydroxybenzoate (nipagin P) were confirmed over the ranges 0.5–30, 5–200, and 10–120 $\mu g.mL^{-1}$, respectively.

4.8.2 Determination of fluocinolone acetonide in pharmaceutical preparations by differential-pulse polarography

A simple differential-pulse polarographic method has been developed for the assay of fluocinolone acetonide in cream, gel and ointment. A 0.03 M solution of tetraethylammonium hydroxide containing 50%*V/V* of methanol at pH 11 was used as the supporting electrolyte. Commonly used preservatives and bases for the preparations were found not to interfere in the determination. A calibration graph was prepared by the method of standard additions. Results obtained using the proposed and official procedures were comparable.

4.8.3 Analysis of creams (objective reference): Quantitative determination of drugs in creams by UV spectrophotometry

The uv absorbing properties of the components of the cream bases as described in the Formulary of the Dutch Pharmacists were investigated. DirectUV spectrophotometric determinations without any clean-up steps appeared to be possible for a number of drugs (*e.g.* tripelennamine HCl,

tretinoin, salicylic acid, methyl salicylate, resorcinol, clioquinol), with the help of a solvent mixture in which the cream samples dissolved completely to yield clear solutions. Correcting for the contribution to theUV absorbance by the preservative is sometimes necessary and can be achieved by measuring the absorbance at two wavelengths. The determination of chlorhexidine, as an example of a basic drug withUV absorbing properties which prevent direct measurements of the solution of the cream samples, could be achieved after removal of the interfering compounds by a simple liquid-liquid extraction.

4.9 Hydrocortisone acetate [34-37]

4.9.1 Optimization and validation of an RP-HPLC method for analysis of hydrocortisone acetate and lidocaine in suppositories.

An RP-HPLC method has been optimized and validated for the simultaneous determination of hydrocortisone acetate and of lidocaine in suppositories. For the method optimization, response surface methodology was applied, and the obtained model was tested using analysis of variance. The optimal separations were conducted on a Beckman-Coulter 150 x 4.6 mm, 5µm particle-size column at 20 °C. The mobile phase was methanol-water (65 + 35, v/v), pH adjusted to 2.5 with 85% orthophosphoric acid, with a flow rate of 1.0 ml/min. UV detection was performed at 250 nm. Phenobarbital was used as an internal standard. The method was validated for selectivity, linearity, precision, and robustness.

4.9.2 Analysis of hydrocortisone acetate ointments and creams by high-performance liquid chromatography.

High-performance liquid chromatographic (HPLC) methods for the analysis of hydrocortisone containing ointments and creams have been investigated. A method which uses a silica column and involves a minimum of sample pre-treatment has been shown to compare favourably with the triphenyltetrazolim chloride method of the British Pharmacopoeia. For hydrocortisone ointments the HPLC procedure provides results of equivalent precision and has advantages with respect to the time taken for each analysis and specificity. Application of the method to the analysis of hydrocortisone creams has been explored and the deviation between the HPLC and colorimetric method requires further investigation.

4.9.3 Optimization of a Selective Liquid Chromatography Procedure for Hydrocortisone Acetate, Hydrocortisone Alcohol and Preservatives in a Pharmaceutical Emulsion.

An accurate, reproducible and specific stability-indicating method for the high performance liquid chromatography (HPLC) assay of hydrocortisone acetate, hydrocortisone alcohol, methyl *p*-hydroxybenzoate and propyl *p*-hydroxybenzoate in a pharmaceutical suspension is described. An investigation of several column phases was undertaken and a Zorbax SB-Phenyl column gave the best selectivity and specificity due to the π-π interactions between the analytes and stationary phase. All the components were fully resolved in less than 15 min under isocratic conditions using UV detection at 254 nm with a water-methanol mobile phase. The stability-indicating method was validated over the linearity range of 25% to 150% of the nominal concentrations of each analyte. Nominal concentrations were hydrocortisone acetate (10% *w/w*), hydrocortisone alcohol (0.2% *w/w* with respect to hydrocortisone acetate), methyl p-hydroxybenzoate (0.1% *w/w*) and propyl p-hydroxybenzoate (0.01% *w/w*) respectively.

4.9.4 Simultaneous determination of methylparaben, propylparaben, hydrocortisone acetate and its degradation products in a topical cream by RP-HPLC.

A novel reversed-phase high-performance liquid chromatographic method with UV spectrophotometric detection was developed and validated for the determination of compounds in topical cream. The method describes determination of active component hydrocortisone acetate (HCA), its degradation products hydrocortisone (HC) and cortisone acetate (occurring in formulation after long-term stability tests) and two preservatives presented in the cream- methylparaben and propylparaben, using dexamethasone as an internal standard. The chromatographic separation was performed on a 5 μm SUPELCO Discovery C18 125×4-mm ID column. The optimised mobile phase for separation of all the compounds consists of methanol, acetonitrile and water (15:27:58, v/v/v), with the analysis time less than 13 min. The method was applicable for routine analysis (assays and stability tests) of active compound HCA, preservatives and degradation products in pharmaceutical product—topical cream Hydrocortizone cream 1%.

4.9.5 Comparison of HPLC and multivariate regression methods for hydrocortisone and lidocaine analysis of pharmaceutical preparation.

A reverse-phase high-performance liquid chromatographic (HPLC) method to determine hydrocortisone acetate, hydrocortisone hemisuccinate and lidocaine is described in this paper. The separation was made in a LichrCART C_{18} column using a methanol-NaH(2)PO(4)/Na(2)HPO(4) (0.1 mol L(-1)) (pH=4.5) buffer solution as a mobile phase in isocratic mode (60:40 (v/v)). The mobile phase flow rate and the sample volume injected were 1 mL min(-1) and 20 micro L, respectively. The detection was made with a diode-array detector measuring at the maximum for each compound. Quantification limits ranging from 0.18 to 0.84 micro g L(-1) were obtained when the peak area was measured. The method was applied in pharmaceutical formulations that were compared with those obtained by through multivariate regression spectrophotometry and micellar capillary electrophoresis (MEKC). HPLC results are in accordance with the results obtained by MEKC. The spectrophotometric method was suitable only for synthetic samples.

4.10 References

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