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Methods of Determination of Levocetirizine Dihydrochloride: A Review

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Summary Different methods have been used for the determination of the antihistaminic drug levocetirizine dihydrochloride (LVC.2HCl) including HPLC, spectrophotometry, potentiometry, titrimetry and other spectral methods of analysis. In this review, we will discuss these methods. This will help the persons dealing with this pharmaceutical compound to choose the suitable method for its determination.

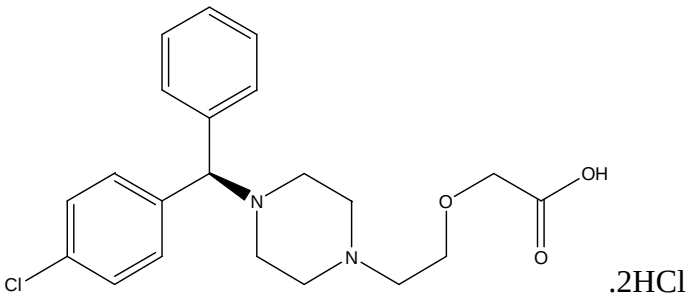
Contents

Introduction

The literature review is a backdrop on which the researcher presents his work. This issue is very important because it gives an idea about the previously published work related to the present work. After a literature survey on the previously reported methods, it was relevant that there are few methods for the

determination of LVC.2HCl. Very few of these methods are spectrophotometric methods and the rest of them are based on other analytical techniques. In this review, we will cover the previously reported methods used for determination of LVC.2HCl. All the previously reported papers of LVC.2HCl are devoted to studies in solution and none of them is devoted to the characterization of its solid complexes although it is a very important issue. In the present review, we will be interested in giving a short summary about the previously published papers about the methods of determination of LVC.2HCl and the other work devoted to the characterization of LVC.2HCl and its compounds.

LVC.2HCl is a 3rd generation antihistaminic drug derived from the second generation drug cetirizine dihydrochloride (CE.2HCl). CE.2HCl has two isomeric structures according to the direction of inversion of polarized light. LVC.2HCl is the L-enantiomer of CE.2HCl. The structure of LVC.2HCl is shown in the following scheme.



.2HCl

Scheme 1 Chemical structure of levocetirizine dihydrochloride

Spectrophotometric methods

Cetirizine hydrochloride was determined by two extractive spectrophotometric methods proposed by Gowda et al.[(1)] The proposed methods are based on the formation of chloroform soluble complexes between cetirizine hydrochloride with bromocresol purple (BCP) and bromophenol blue (BPB). The absorbance was measured at 409 and 414 nm for BCP and BPB, respectively. Under experimental conditions the reagent blank showed

negligible absorbance. Beer's law is obeyed over concentration ranges of 1.0-16.0 and 1.5-21.0 µg/mL for BCP and BPB, respectively. The drug-reagent ratio was found to be 1:1 as evaluated from Job's method. The equations for representative calibration curve are $A_{409\ nm}$ = 0.026 C + 0.1463 and $A_{414\ nm}$ = 0.025 C + 0.0472 for BCP and BPB, respectively. The RSD value was found to be less than 1.0%. Sandell's sensitivity was determined to be 28.5 and 32.2 ng/cm^2 for BCP and BPB, respectively.

El Walily et al.[2] reported two spectrophotometric methods for the determination of cetirizine dihydrochloride in tablets. The first method is based on derivative spectrophotometry. In this method, cetirizine was determined by the measurement of its first (^{1}D) and second (^{2}D) derivative amplitudes at 239 nm (peak) and 243-233 nm (peak-to-trough), respectively. Beer's law is obeyed in the concentration ranges of 1.2-10.0 and 0.8-10.0 µg/mL for first (^{1}D) and second (^{2}D) derivative measurements, respectively. The second method is based on the colorimetric determination of cetirizine dihydrochloride through the formation of a colored chromogen from the reaction between cetirizine sodium salt in polar solvent (DMF) and chloranil. The absorbance was measured at 556 nm. Beer's law is obeyed in the concentration range 120-250 µg/mL.

A simple, rapid and sensitive spectrophotometric method has been developed for the assay of ceterizine hydrochloride in bulk drug and its pharmaceutical preparations.[3] This method is based on the ion-pair complex reaction between the drug and alizarin red S in Clarks–Lubs buffer. The chromogen being extractable with chloroform could be measured quantitatively at 440 nm. All variables were studied to optimize the reaction conditions. Regression analysis of Beer’s Law plot showed good correlation in the concentration range 2.5–22 mg/mL. The method has a detection limit of 0.1328 mg/mL. The proposed method has been successfully applied for the analysis of the bulk drug and its dosage forms such as tablets and syrups. No interference was observed from common pharmaceutical adjuvants.

A spectrophotometric method for the determination of cetirizine was proposed using BCP.[4] The method is based on the formation of chloroform-extractable complex with bromocresol purple. The absorbance of the ion-pair complex is measured at 410 nm. Mclivaine's citric acid phosphate buffer pH 2.6 was used throughout the study. Some organic solvents were applied for extraction such as chloroform, toluene, chlorobenzene and methylene chloride. It was found that chloroform is the most ideal solvent that yields maximum absorbance. The developed color was stable for more than one hour. Beer's law is obeyed over the concentration range of 5-10 µg/mL. The molar absorptivity of the formed ion-pair is 2.85×10^4 L/mol cm.

A spectrophotometric method based on the formation of ion-pair complex with eosin was reported.[4] The formed reddish-orange chromophore has a λ_{max} at 540 nm. The ion-pair formation was optimized using eosin and Mclivaine's citric acid phosphate buffer of pH 3.0 was used for complete color development. Various surfactants such as sodium lauryl sulphate, methyl cellulose, Tween 20 and Tween 80 were tried to increase the solubility of the formed complex and the highest sensitivity was obtained using methyl cellulose solution. The developed color is stable for at least one hour. Beer's law is obeyed in the concentration range of 8-24 µg/mL. The formed ion-pair has a molar absorptivity of 1.72×10^4 L/mol cm.

Determination of cetirizine was carried out using a method developed by Gazy et al.[4] using base-catalyzed condensation of mixed anhydrides of organic acids. The tertiary amino group of the drug acts as the basic catalyst. The organic acids used were acetic and citric acids. The formed colored condensation products were measured spectrophotometrically. 30 minutes in boiling water bath gave maximum sensitivity. The formed product has a λ_{max} at 543 nm. Beer's law range is very narrow (3-7 µg/mL), but the molar absorptivity of the formed product was high to some extent (8.48×10^4 L/mol cm).

A spectrophotometric method for the determination of cetirizine hydrochloride is based on the measurement of the chloride of the drug.[5] The determination was carried out by addition of different amounts of cetirizine hydrochloride to a fixed concentration of mercury(II)-diphenylcarbazone complex and the decrease in absorbance of mercury(II)-diphenylcarbazone complex, consequent to the replacement of diphenylcarbazone of the complex by the chloride ion of the drug, was measured at 540 nm. Beer's law is obeyed in the concentration range 0-100 µg/mL. The optimum pH of determination lies between 3.3 and 3.5. The formate-formic acid buffer of pH 3.4, which neither complexes mercury (II) nor contains chloride ions was used.

A derivative spectrophotometric method is developed for the assay of the binary mixture of pseudoephedrine with cetirizine.[6] The method is based on the use of the first derivative of the ratio spectrum. The stored absorption spectra of standard solutions of cetirizine were divided (amplitude by amplitude) by the absorption spectrum of a standard solution of 384 µg/mL pseudoephidrine to obtain the corresponding ratio spectra, then the first derivative of the ratio spectra were calculated with $\Delta\lambda$ = 2 nm. The obtained spectra showed that cetirizine can be determined at three different wavelengths (225.4, 234.5 and 238 nm), from which λ_{225} nm was chosen as it has the highest response. The concentration of the other components was determined from its respective calibration graph treated similarly. The absorbances of the standard and sample solutions were recorded within the wavelength range 200-300 nm. The influence of $\Delta\lambda$ for obtaining the first derivative of the ratio spectra and the effect of the divisor concentration on the calibration graphs were studied.

Choudhari et al.[7] presented a ratio derivative spectrophotometric method for the simultaneous determination of montelukast sodium (MON) and LVC.2HCl in pharmaceutical preparations. The absorption spectrum of a mixture of the two drugs is divided by the absorption spectrum of one of the two components and the first derivative of the ratio spectrum is obtained. The concentration of active compounds is then determined from calibration graph

obtained by measuring amplitude at points corresponding to minima or maxima. Wavelength 250.4 nm was selected for the quantification of montelukast sodium in MON + LVC.2HCl mixture and 238.4 nm was selected for the quantification of LVC.2HCl in MON + LVC.2HCl mixture. The amounts of MON and LVC.2HCl in tablets are calculated using the following equations:

At 250.4 nm:

$$C_{MON} = d/d\lambda\ [A_{MON} / A_{LVC.2HCl}] - \text{Intercept (c)} / \text{Slope (m)} \quad (1)$$

At 238.4 nm:

$$C_{LVC.2HCl} = d/d\lambda\ [A_{LVC.2HCl} / A_{MON}] - \text{Intercept (c)} / \text{Slope (m)} \quad (2)$$

Beer's law is obeyed in the concentration range 4-12 and 2-6 µg/mL with correlation coefficients of 0.999 and 0.997 for MON and LVC.2HCl, respectively. For MON, the recovery study results ranged from 99.79 to 100.68% with %RSD values ranging from 0.394 to 0.777. For LVC.2HCl, the recovery results ranged from 99.44 to 100.2%, with %RSD values ranging from 0.425 to 0.808. The accuracy and reproducibility are evident from the data as results are close to 100% and the standard deviations are low.

Determination of LVC.2HCl in a mixture with ivermectin in bulk and in combined tablet dosage forms was carried out by Ashok and Chandra.[8] A UV method based on the application of the simultaneous equation was suggested. All of these methods utilize (1:1) acetonitrile and water as a solvent. LVC.2HCl shows an absorption maximum at 230 nm, while the UV spectrum of ivermectin shows a maximum at 245 nm. Beer's law is obeyed in the concentration ranges of 1.0-6.0 and 1.2-7.2 µg/mL for LVC.2HCl and ivermectin, respectively. The accuracy of the methods was found to be 97.75-100.9% for LVC.2HCl and 98.85-99.43% for ivermectin, respectively. The RSD was 0.5974 and 0.4096%.

Wahba et al.[9] developed a spectrofluorimetric method for the determination of some H1-receptor antagonists including cetirizine dihydrochloride. The proposed method is based on the reaction of the drug with 2-cyanoacetamide to give highly fluorescent derivatives in alkaline medium.

The formed derivatives are measured at 365 nm after excitation at 312 nm. The method was applied for the determination of cetirizine dihydrochloride in spiked human plasma samples and was used to reveal the pharmacokinetic characters in a healthy volunteer treated with oral administration of the different doses of the drug. Kinetic studies on the acidic, alkaline, oxidative and ultraviolet degradation products were carried out. The limit of detection (LOD) and limit of quantification (LOQ) were 2.7 and 8.3 ng/mL, respectively.

Recently, Basavaiah et al.[10] proposed a new method for the determination of the cited drug in pharmaceuticals based on charge-transfer (C.T.) reactions. The drug (n-electron donor) interacts with picric acid, 2,4-dinitrophenol (π-acceptor) or iodine (I_2) as a σ-acceptor to give highly colored radical anion species. The quantification of the colored products was performed by measuring their absorbance at 420 nm with both 2,4-dinitrophenol and picric acid methods and at 375 nm with I_2. Beer's law concentration ranges are 1.2-24, 1.6-32 and 2.4-48 µg/mL, LOD was found to be 0.20, 0.39 and 0.51 µg/mL and LOQ is 0.61, 1.17 and 1.53 µg/mL for 2,4-dinitrophenol, picric acid and I_2-methods, respectively.

A validated simple, rapid and selective spectrofluorimetric method was developed for the determination of cetirizine dihydrochloride.[11] C.T. reaction with some π-acceptors such as p-chloranilic acid (CLA), 2,3-dichloro-5,6-dicyano-p-benzoquinone (DDQ) or tetracyanoethylene (TCNE) to give highly fluorescent derivatives is the principle of the method. The fluorescence intensity-concentration plots were rectilinear in the concentration ranges of 0.5-7.0, 0.5-6.0 and 0.2-4.0 µg/mL with CLA, DDQ and TCNE, respectively. The factors affecting the reaction were carefully studied. The stoichiometry of the reactions was found to be 1:1 in all cases.

Cetirizine was determined in bulk drug, dosage formulations and human serum.[12] The method involves simultaneous use of linear regression function obtained by plotting the concentration versus absorbance in the working concentration range. To validate the method, analytical parameters such as

selectivity, precision and accuracy were studied for different brands of tablets and have been evaluated statistically to assess its application. The goodness-of-fit (r^2) was found to be 0.999. The LOD and LOQ were studied at two pH levels (pH 1.2 and pH 7.4). LOD was found to be 0.04 and 0.03 µg/mL for cetirizine at pH 1.2 and pH 7.4, respectively. LOQ values were 0.14 µg/mL at pH 1.2 and 0.11 µg/mL at pH 7.4 for raw material. The LOD and LOQ values in serum were 0.04 and 0.15 µg/mL, respectively.

A spectrofluorimetric method was developed for the determination of LVC.2HCl.[13] Ce(IV) was used to oxidize the drug in presence of sulphuric acid. The resulting Ce(III) was monitored at λ_{ex} 249 nm and λ_{em} 354 nm. The variables affecting the reaction such as Ce(IV) concentration, sulphuric acid concentration, heating time, temperature and diluting solvents were studied. The calibration graphs were rectilinear in the concentration range of 0.05-5.0 µg/mL. The LOD and LOQ were found to be 0.01 and 0.05 µg/mL, respectively. Interference studies were carried out using common ingredients such as glucose, sucrose, lactose, citric acid and propylene glycol. Standard addition method was applied and the obtained recovery was 99.36-100.27% from their corresponding dosage forms.

A spectrophotometric method was used to determine LVC.2HCl and montelukast sodium in combined pharmaceutical dosage forms.[14] The method is based on using AUC (area under the curve) spectrophotometric method. The principle of this method is “The area under the two points on the mixture spectra is directly proportional to the concentration of the component of interest”. The selected areas were 263.6 to 293.6 nm and 222 to 242 nm for determination of montelukast sodium and LVC.2HCl, respectively. Beer-Lambert’s law is obeyed in the concentration range of 5-30 µg/mL for both drugs. The recovery values were found near to 100%. The validation of the method was carried out according to the ICH guidelines.

Methyl orange was used for the spectrophotometric quantification of cetirizine dihydrochloride in its pure form and different pharmaceutical

preparations.[15] This method is based on the formation of ion-pair complex with methyl orange at pH 4.0, which produces a yellow color after chloroform extraction. The formed ion-pair complex exhibited a maximum absorbance at 424.5 nm. Beer-Lambert's law is applicable over the concentration range of 2.5-20 µg/mL. The various parameters affecting the color development were studied according to the ICH guidelines. The detection and quantification limits were found to be 1.0 and 3.0 µg/mL, respectively. No interference was observed for common pharmaceutical excipients.

The use of derivative spectrophotometry has attracted a great attention because it has solved the problem of interference in simultaneous determination of drugs. A first derivative (^{1}D) ultraviolet spectrophotometric method was developed for the determination of cetirizine and montelukast in their pharmaceutical dosage forms.[16] Cetirizine was measured at 217 nm and montelukast was measured at 335 nm. The calibration curves were rectilinear in the concentration ranges 2-20 µg/mL (r^2 = 0.9991) for cetirizine and 6-28 µg/mL (r^2 = 0.9976) for montelukast. The relative standard deviation was found to be <0.4. The calculated LODs were 0.1 and 0.01 µg/mL for cetirizine and montelukast, respectively.

Cetirizine hydrochloride was determined in combined tablets of cetirizine hydrochloride and ambroxol hydrochloride using a UV-Vis spectrophotometric method.[17] The simultaneous equation method was used to indicate the concentration of the two drugs. The calibration curves were rectilinear in the concentration ranges 5-60 and 5-70 µg/mL for cetirizine hydrochloride and ambroxol hydrochloride, respectively. LOD and LOQ are 3.54 and 10.73 µg/mL for cetirizine hydrochloride and 6.88 and 20.85 µg/mL for ambroxol hydrochloride, respectively.

A spectrophotometric method has been developed for the simultaneous estimation of ambroxol hydrochloride and LVC.2HCl.[18] The method involved solving simultaneous equations based on measurement of absorbance at two wavelengths 242 and 231 nm, the λ_{max} of ambroxol hydrochloride and

LVC.2HCl, respectively. Beer's law is obeyed in the concentration range 10-50 µg/mL and 8-24 µg/mL for ambroxol hydrochloride and LVC.2HCl, respectively. Results of the method were validated statistically and by recovery studies.

Chromatographic methods

No doubt that, chromatographic methods of analysis, especially HPLC, are considered one of the most applicable techniques for determination of pharmaceutical compounds. This may be due to the high sensitivity of these methods, the capability of these methods to determine the compound of interest in complicated matrices, such as biological samples, without pretreatment of the samples and the low detection limits they have. LVC.2HCl was determined using different chromatographic methods; the most of them are HPLC methods. The difference between these methods may depend on the detection system, the composition of the mobile phase and/or the column used in the determination procedure.

A liquid chromatography-tandem mass spectrometric method (LC-MS/MS) was described for LVC.2HCl quantification in human plasma.[19] Sample preparation was made using a fexofenadine addition as an internal standard (IS), liquid-liquid extraction using cold dichloromethane and dissolving the final extract in acetonitrile. LVC.2HCl and fexofenadine (IS) were injected in a C18 column and the mobile phase composed of acetonitrile:water:formic acid (80:19:0.1, v/v/v) and monitored using positive electrospray source with tandem mass spectrometry analysis. The selected reaction monitoring (SRM) was set using precursor ion and product ion combinations of m/z 389>201 for LVC.2HCl and m/z 502>467 for fexofenadine. The LOQ and the dynamic range achieved were 0.5 ng/mL and 0.5-500.0 ng/mL, respectively. Biological samples spiked with LVC.2HCl were injected in decreasing concentrations until the lowest concentration quantified with precision and accuracy, expressed by R.S.D. lower than 20%. The method was highly selective, with no interfering compounds or significant ion suppression from endogenous substances observed at the retention times for

LVC.2HCl and fexofenadine. The RSD was less than 7%. The relative error (RE) of the mean of the measured concentrations ranged from 8.60 to 3.57%. The determination coefficients (R_2) were greater than 0.997 for all curves. The recovery of LVC.2HCl, determined at three different concentrations (1.50, 200.0 and 400.0 ng/mL), were 82.97, 85.78 and 95.04%, respectively; the overall average recovery was 87.93%. This method allows an accurate, precise and reliable measurement of LVC.2HCl concentrations in human plasma for up to 48 h after a single oral dose of 5 mg administered to healthy volunteers.

An HPLC assay for simultaneous determination and quantification of cefpirome and LVC.2HCl in their pure form, in pharmaceutical formulations and in human plasma without changing the chromatographic conditions was described.[20] The used column was a prepacked Nucleosil 120, C18 (5 µm, 12.5±0.46 mm). CH_3CN:H_2O (75:25, v/v) was used as a mobile phase with a flow rate of 1 mL/min with UV detection at 232 nm for monitoring the effluent. A number of other brands of C18 columns was also employed which had a significant effect on the separation. This method has been validated over the concentration range of 0.5-50.0 µg/mL. The LOD and LOQ for cefpirome and LVC.2HCl in pharmaceutical formulations and in serum range from 0.24 to 1.31 µg/mL. Analytical recovery from human plasma was more than 98% and the within and between-day relative standard deviation was less than 3.1%. A seven-point calibration curve was constructed with working standards and was found to be linear (r^2>0.999) for each of the analytes over their calibration ranges. The linearity was evaluated by the least squares regression method and the proposed method was evaluated by its correlation coefficient and intercept values. No change was observed in the chromatogram of LVC.2HCl in the presence of common excipients.

A TLC method was presented for determination and chiral discrimination of LVC.2HCl.[21] This method is based on the enantioseparation of cetirizine on silica gel TLC plates using different chiral selector, namely hydroxyproyl-β-cyclodextrin (HP-β-CD), chondroitin sulphate or vancomycin HCl. The chromatographic tank was saturated with the mobile phase for 10 min

before development of the plates. For separation and detection, 20 µL of racemic cetirizine and 10 µL of standard solution of LVC.2HCl were applied as separate compact spots 20 mm apart and 10 mm from the bottom of the TLC plates using a 25 µL Hamilton micro syringe. The chromatograms were developed up to 8 cm in the usual ascending way, air dried and visualized under UV wavelength of 254 nm or by exposure to iodine vapours.

One of the methods used for the determination and chiral discrimination of cetirizine is an HPLC method based on the stereoselective separation of cetirizine and quantitative determination of its eutomer (R)-levocetirizine on a monolithic C18 column using hydroxyproyl-β-cyclodextrin as a chiral mobile phase additive.[21] The resolved peaks of (R)-levocetirizine and (S)-levocetirizine were confirmed by further mass spectrometry. A mixture of acetonitrile-methanol-tetrahydrofuran-0.05 mol/L potassium dihydrogen orthophosphate containing 1 mM HP-β-CD, 50:25:5:120, (v/v) pH 5.2±2 was used as the mobile phase. The pH was adjusted with phosphoric acid and/or triethanolamine. The HPLC flow rate was set at 1 mL/min and a UV wavelength of 230 nm was selected for detection. All analysis was performed at 25±2 ºC with a sample injection volume of 20 µL. The resolved fractions corresponding to (R)-levocetirizine and (S)-dextrocetirizine were collected and freeze dried. The samples were dissolved in a 50% solution of acetonitrile in water, 10 µL of each sample was injected to the mass spectrometer and the spectra were recorded. For quantitative determination of (R)-levocetirizine aliquots of standard solution (1 mg/mL) equivalent to 0.025-2.0 mg were transferred into 10 mL volumetric flasks and made up to volume with the mobile phase. Triplicate 20 µL injections were made of each concentration. The average peak areas were calculated and plotted versus concentrations. A linear relationship was obtained and the regression equation was recorded.

A sensitive HPLC method was developed and validated for the determination of LVC.2HCl related substances in solid oral formulation.[22] The mobile phase used for the elution and separation of LVC.2HCl and its related substances is composed of acetonitrile and dilute sulphuric acid. Lichrocarrt

Si60, 250 × 4.0 µ (cartridge column) was used at 0.8 mL/min flow rate to achieve good resolution of the analyte and its impurities. LVC.2HCl was exposed to various stress conditions such as acid, base, oxidation and thermal degradations along with placebo and formulation, but it was found to be stable under all stress conditions. The detector linearity was established for concentrations ranging from 0.06 to 0.60 µg/mL for LVC.2HCl and 0.03 to 0.30 µg/mL for its impurities with a correlation coefficient of 0.9999. The LOD and LOQ are found to be in a range of 0.01 and 0.06 µg/mL for LVC.2HCl and 0.005 and 0.03 µg/mL for related substances, respectively. The drug was extracted from the tablet formulation of 5.0 mg label claim by using the mobile phase as diluent. Sample was diluted with mobile phase to achieve final working concentration. This method was found to be precise with six sample preparations for the quantification of LVC.2HCl and its impurities. The %RSD of LVC.2HCl and its total impurity in six sample preparations was found to be 2.0 for assay and 5.0 for impurity. The recovery range for all impurities was found to be between 96-106% with %RSD of 0.65-2.87.

A stability indicating reverse phase high performance liquid chromatography (RP-HPLC) has been developed for the determination of LVC.2HCl and pseudoephedrine sulfate in their pharmaceutical formulation.[23] In the chromatographic system, a Cosmosil C8, 250 × 4.6 mm, 5 µm column was used. The mobile phase (A) consists of potassium dihydrogen phosphate buffer 0.05 mol/L and 1-octane sulphonic acid sodium salt 0.25% and the pH was adjusted to 3.0 with orthophospheric acid. Mobile phase (B), acetonitrile, gradient elution at flow rate of 1 mL/min and column temperature at 40 ºC was used. Detector wavelength was kept at 242 nm which is the isobestic point using a photodiode array detector. This method is very specific as no interference occurred from the drug excipients in the formulation. The calibration graphs were found to be linear in the concentration ranges 10-200 µg/mL for LVC.2HCl and 360-7200 µg/mL for pseudoephedrine sulfate. The correlation coefficient of the two drugs is 0.9999. Precision was expressed as

percent relative standard deviation (%RSD<2). The LOQs are 0.036 and 1.4 for LVC.2HCl and pseudoephedrine sulfate, respectively.

Enantioselective method has been developed and validated for the determination of LVC.2HCl in human plasma.[24] This assay was performed using normal-phase liquid chromatography coupled to tandem mass spectrometry with an atmospheric pressure chemical ionization (APCI) interface in the positive ion mode. The separation is performed on a CHIRALPAK AD-H column using an isocratic mobile phase. The mobile phase is composed of a mixture of n-hexane, ethyl alcohol, diethylamine, and acetic acid (60:40:0.1:0.1, v/v/v/v). The internal standard used was in the form of LVC-D_8. Both LVC and internal standard were detected by multi-reaction monitoring (MRM). A simple two step extraction by protein precipitation using acetonitrile followed by liquid-liquid extraction with an n-hexane-dichloromethane mixture (50:50, v/v) was used to prepare the samples. The standard curve for LVC.2HCl was linear (r^2>0.995) in the concentration range 0.5-300.0 ng/mL. The recovery was between 97.0 and 102.2% at low, medium and high concentrations. The LOQ was 0.5 ng/mL. Finally, the proposed method was successfully applied to the study of enantioselective oral pharmacokinetics of LVC.2HCl in healthy Korean volunteers.

A sensitive HPLC method was developed for the determination of LVC.2HCl and amproxol in human plasma and urine samples.[25] The internal standard used in this method is nebivolol. Plasma samples were prepared by extraction in methylene chloride and a mixture of diethylether (80:20, v/v). A supelcosil TMLCABZ (50 × 4.6 mm, 5 µm particle size) column and a mobile phase 1 comprising acetonitrile-phosphate buffer (pH 3.5; 20 mM) (20:80, v/v) were used in the assay. The eluate of LVC.2HCl and amproxol were separated to an analytical Kromasil C8 micropore column (50 × 0.3 mm, 5 µm particle size) via a column switching device. A Kromasil C18 analytical column (250 × 2.1 mm, 5 µm particle size) was used as a separation column. Mobile phase 2 consisting of acetonitrile-triethylamine (0.5%) in phosphate buffer (pH 3.5, 20 mM) (55:45, v/v) was used for the compound elution. The eluents are detected

at 230 nm with photodiode array detector. The total run time was 25 min for a sample. The method is linear in the range 2.0-450 and 7.0-300 ng/mL for LVC.2HCl and amproxol, respectively; in plasma. In urine samples, the range is 1.0-500 and 5.0-400 ng/mL for LVC.2HCl and amproxol, respectively. Intra-day and inter-day precision of LVC.2HCl and amproxol were below 15%. The accuracy ranges from 94.0 to 101.6% and 91.0 to 100.2% for LVC.2HCl and amproxol, respectively.

Recently, simple, accurate and precise RP-HPLC method was introduced for estimation of LVC.2HCl and montelukast sodium in tablet dosage form.[26] This method was carried out using water HPLC system on a L7 column (Hypersil Gold: 250 × 4.6 mm, 5 µm). The mobile phase is composed of a mixture of 0.05 mol/L potassium dihydrogen phosphate buffer of pH 7.5 and methanol in the ratio 20:80 v/v. A flow rate of 1.2 mL/min was used. All determinations were performed at a constant column temperature of 35 ºC with a lode of 10 µL. The detection was carried out at 225 nm. The retention times of LVC.2HCl and montelukast were found to be around 3.2 and 4.2 min, respectively. The calibration curves are linear in the range 10-260 µg/mL for LVC.2HCl and 10-350 µg/mL for montelukast. The RSD for assay results of six determinations is less than 1% for both components which confirms the high degree of precision of the method. To evaluate the robustness of the method, the mixed standard solution containing 0.1046 mg/mL LVC.2HCl and 0.1994 mg/mL montelukast was injected in replicate under varied chromatographic conditions and the SD of the retention time of each analyte was calculated. The method was found to be robust as the slight variation in the flow rate and in the detection wavelength did not lead to any change in the retention time of the peak of interest. The values of LOD = 3.3 σ/s (where σ is the standard deviation and s is the slope of the calibration curve), were found to be 2.26 and 2.41 µg/mL for LVC.2HCl and montelukast, respectively. The values of LOQ = 10 σ/s were found to be 6.85 µg/mL for LVC.2HCL and 7.3 for montelukast.

Two chromatographic methods have been developed for the simultaneous determination of LVC.2HCl and montelukast sodium in bulk drug and in tablets.[27] The first method is a high performance thin layer chromatographic (HPTLC) separation followed by densitometric measurements on normal phase silica gel 60 F254. A mobile phase consisted of toluene:ethylacetate:methanol:ammonia (2.5:7:2.5:1, v/v/v/v) was selected. The mixed standard stock solution containing 1.0 mg/mL of LVC.2HCl and 2.0 mg/mL montelukast sodium was spotted on the HPTLC plate and run in the solvent system. The mobile phase was run up to a distance of 8.0 cm which takes approximately 25 min for complete development of the thin layer chromatography plate. The second method is an HPLC separation on a BDS hypersil C18 column using disodium hydrogen phosphate buffer (0.02 mol/L):methanol (25:75, v/v). pH was adjusted to 7.0 with orthophospheric acid as a mobile phase. The separation was carried out at ambient temperature with a flow rate of 1.0 mL/min. The retention times (t_R) for LVC.2HCl and montelukast sodium were found to be 3.558±0.03 and 7.450±0.04 min, respectively.

An HPLC method[28] was developed for the determination of ambroxol HCl and LVC.2HCl in tablets. The solvent used is a mixture of acetonitrile, methanol and water in the ratio 10:20:70 (v/v/v). The column which was used in the HPLC procedure is a Phnomenex Gemini C18 (25 cm× 4.6 mm i.d., 5µ) at a flow rate of 1.0 mL/min. Detection was carried out at 244 and 230 nm. The retention time of ambroxol HCl and LVC.2HCl was 4.2 and 9.3 min, respectively. This method was used for the determination of both drugs in combined tablets.

A stereoselective method or the determination of cetirizine was reported.[29] The method is based on chiral separation of cetirizine enantiomers. α1-Acidglycoprotein based chiral stationary phase (AGP-CSP) was monitored with UV at 230 nm to determine the enantiomers. 10 mmol/L phosphate buffer (pH 7.0)-acetonitrile (95:5, v/v) was chosen as the mobile phase. The optimum extraction efficiency was obtained at pH 5 with ethyl acetate as the solvent of

choice. Extraction recovery of (+)-cetirizine was 73.2 (1.3%) and that of (-)-cetirizine was 71.2 (2.0% (n=5)). The linearity of the method was evaluated in the concentration range of 2.5 to 200 µg/mL for cetirizine enantiomers. The correlation coefficients were better than 0.997 (n=5). The observed enantioselectivity (α) was 2.0.

Kuroda et al.[30] used precolumn fluorescence labeling method for the simultaneous determination of hydroxyzine and cetirizine in human serum. This method is highly selective and sensitive. Haloperidol was used as an internal standard. This method is based on the fluorescence labeling of both drugs with a fluorescent arylboronic acid 4-(4, 5-diphenyl-1H-imidazol-2-yl)phenylboronic acid followed by separation on silica column using a mobile phase consisting of acetonitrile and water (90:10, v/v) containing triethylamine and acetic acid. Liquid-liquid extraction was used to extract both drugs from human serum. The linearity range was 0.025-2.00 µg/mL for hydroxyzine and cetirizine. The LOD (S/N = 3) was found to be 0.01 and 0.005 µg/mL for hydroxyzine and cetirizine, respectively.

Eighteen antihistamine drugs including cetirizine were simultaneously screened and quantified in blood samples using liquid chromatography-tandem mass spectrometry (LC-MS/MS).[31] Liquid-liquid extraction of the basic antihistamines followed by a second extraction of the acidic antihistamines was performed to the samples prior to the determination procedures. The recoveries were 43-113% for basic drugs and 23-66% for acidic drugs. The combined extracts were run by liquid chromatography on C18 reversed phase column using acetonitrile-ammonium acetate mobile phase of pH 3.2. Screening was performed using multiple reaction monitoring (MRM). The LOQs varied between 0.0005 and 0.01 µg/mL in blood and were lower than the therapeutic concentrations (C_{max}). The intra-assay relative standard deviations were better than 10% and the inaccuracy varied between 39% for levocabastine and 5% for cyclizine, The majority of the values being <20%.

Titrimetric methods

A titrimetric method for the determination of cetirizine hydrochloride was pointed based on the measurement of the chloride of its hydrochloride.[5] The chloride content of the drug is determined by titration with mercury(II) using diphenylcarbazone-BTB (BTB) as indicator. The stoichiometric study revealed that the drug-titrant ratio in the complex formed was 1:2. This method could be used for the determination of 4-15 mg of cetirizine hydrochloride using (9.4×10^{-3}-3.5×10^{-3} mol L^{-1}) mercury nitrate solution.

Electrochemical methods

Patil et al.[32] constructed a multi-walled carbon nanotube (MWCN) film-modified glassy carbon electrode (GCE) for the determination of cetirizine dihydrochloride using cyclic voltammetry (CV). The subtle electronic properties suggest that carbon nanotubes (CNTs) have a capability to promote electron transfer reactions and improve sensitivity and thus they are widely used as electrodes. On the other hand, they significantly enhance the oxidation peak current of cetirizine dihydrochloride. The oxidation peak current is linearly proportional to the concentration of the drug in the range 5.0×10^{-7} to 1.0×10^{-5} mol/L. The detection limit was found to be 7.07×10^{-8} mol/L. The MWCN-modified GCE was activated in the potential range 0-1.60 V in presence of phosphate buffer (pH 3.0, ionic strength = 0.2 mol/L) until stable cyclic voltammograms were obtained. Then electrodes were transferred into another cell of phosphate buffer (pH 3.0, ionic strength = 0.2 mol L^{-1}) containing proper amount of cetirizine dihydrochloride. After accumulating for 180 s at open circuit under stirring and following quiet for 10 s, potential scan was initiated and cyclic voltammograms were recorded between 0.60 and 1.40 V, with a scan rate of 50 mV/s. All measurements were carried out at room temperature of 25±0.1 ºC. When the method was used for tablet analysis, the results are in good agreement with the contents on the label. For 10 mg tablets, the detected content was 9.85 mg per tablet with 98.5% recovery.

Cetirizine was determined potentiometrically using membrane sensors of conventional electrode (CE) and carbon paste electrode (CPE) types.[33] The electrodes have been prepared based on β-cyclodextrin as ionophore and potassium tetrakis [3,5-bis(trifluoro-methyl)phenyl]borate (KTFPB) as anionic membrane additive. The linear ranges of the prepared sensors have mean calibration graph slopes of 60.2 and 57.4 mV per decade at 25 ºC for CE and CPE, respectively. The concentration ranges were 1×10^{-1}-5×10^{-6} mol/L for CE and 1×10^{-1}-7×10^{-6} mol/L for CPE. The effect of composition, usable pH range, life span and response time was studied. The sensors were used for the determination of the drug in spiked human urine samples and the mean average recovery was 100.4% with mean precision of ±0.4. This method has the advantages of stability, short response time, selectivity, accuracy and low cost.

Other methods

A ^{1}H-NMR technique was used for characterization of cetirizine and (R)-levocetirizine.[21] Cetirizine dihydrochloride and (R)-LVC.2HCl, 10 mg of each, were dissolved in 600 µL methanol-d4 in eppendorf tubes. The standard ^{1}H-NMR spectra of these solutions were recorded. The chemical shifts were referenced to the centre of the peaks of the solvents residual signal of methanol at 3.31 ppm (^{1}H-NMR). Each sample was measured in triplicates. The aromatic region of the spectrum shows three distinct multiplets, one multiplet with an integral of one and two multiplets with an integral of four. The three multiplets are shifted downfield in the spectrum of the racemate relative to the spectrum of the pure (R)-levocetirizine. The chemical shifts of the centre of the three multiplets are 7.34, 7.40 and 7.62 ppm in the racemate and 7.36, 7.42 and 7.67 ppm in the (R)-levocetirizine.

Sekar used capillary zone electrophoresis for the simultaneous determination of cetirizine dihydrochloride, paracetamol and phenylpropanolamine hydrochloride in tablets.[34] The most suitable

background electrolyte was found to be 10 mmol/L sodium tetraborate solution of pH 9.0. An uncoated fused-silica capillary of a total length of 76 cm (effective length of 64.5 cm) was used for separation. The applied voltage was 20 kV (current produced ≈ 21 μA). All the analytes were completely separated within 10 min. Detection was performed at 195 nm using a UV detector. Ibuprofen was used as an internal standard. The calibration curves were rectilinear in the concentration ranges of 2-50 μg/mL (r^2 = 0.9982), 10-1000 μg/mL (r^2 = 0.9978) and 10-100 μg/mL (r^2 = 0.9986) for cetirizine dihydrochloride, paracetamol and phenyl-propanolamine hydrochloride, respectively. The recovery values were found to be ≥98.6%. No interference from drug excipients was observed. LOQ was 2.0, 2.0 and 4.0 μg/mL of cetirizine dihydrochloride, paracetamol and phenylpropanolamine hydrochloride, respectively.

Conclusion

This review summarizes a brief literature survey on the methods used for the determination of CE.2HCl and its enantiomers. The methods were categorized according to the techniques of chemical analysis used. It is obvious that, there are many chromatographic methods for the determination of the cited drug, two potentiometric methods and only one titrimetric method was found.

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