Simultaneous Estimation of Quinapril Hydrochloride and Hydrochlorothiazide from Pharmaceutical Formulation by Using UV, IR and RP-HPLC

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The objective of present investigation was to develop and validate assay methods for simultaneous estimation of quinapril hydrochloride and hydrochlorothiazide in combined dosage form by using three different analytical approaches viz. UV, FT-IR and RP-HPLC. Drugs were estimated by dual wavelength method and calibration curve methods by using UV and FT-IR spectrophotometer, respectively. The stability indicating assay method was developed by using HPLC. The stress degradation study was carried out according to International Conference on Harmonization (ICH) guideline Q1A (R2). Chromatographic separation was carried out on HIQ-Sil (150 × 4.6 mm, 5 μm) column using 0.01 M KH₂PO₄ buffer (pH 3.5, adjusted with orthophosphoric acid); methanol as mobile phase in gradient elution mode.

Keywords: Dual wavelength, DRIFTS, Hydrochlorothiazide, Quinapril hydrochloride, RP-HPLC.

INTRODUCTION

Hypertension is an important contributor to the risk of cardiovascular disease and death, yet success in achieving blood pressure control has been limited. Most of the patients require 2 or more medications to control their blood pressure. Current guidelines for treatment of hypertension also support the first-line use of combination therapy in many patients. Initiating therapy with a RAS (renin angiotensin system) inhibitor-based combination can reduce blood pressure and cardiovascular risk and may be more effective for majority of patients than traditional combinations such as a β-blocker with a diuretic¹. Quinapril hydrochloride (QUINA) is an angiotensin-converting enzyme inhibitor (ACE inhibitor) used in the treatment of hypertension and congestive heart failure. Chemically it is [3S-[2[R*(R*)],3R*]](S)-2-[(S)-N-[(S)-1-carboxy-3-phenyl-propyl]alanyl]-1,2,3,4-tetrahydro-isoquinoline carboxylic acid, 1-ethyl ester, monohydrochloride. Monograph of QUINA is included in USP² [Fig. 1 (a)].

Hydrochlorothiazide (HCTZ) is a thiazide diuretic most commonly prescribed for hypertension and mild heart failure. Chemically it is 6-chloro-3,4-dihydro-2H-1,2,4-benzothiazine-7-sulphonamide-1,1-dioxide. Monograph of HCTZ is included in IP³, EP⁴ and USP⁵.

Currently both drugs are available in combination under brand names of Accupro Comp, Accuzide, Koretic, Co-Quinapril

and Accuretic. Various UV-visible spectrophotometric (UV)⁶⁻⁸, high performance thin layer chromatographic (HPTLC)⁹ and high performance liquid chromatographic (HPLC)¹⁰⁻¹⁴ methods have been reported for quantitative determination of QUINA and HCTZ in standalone and combination products. The literature also shows few methods reported for simultaneous estimation of QUINA and HCTZ in fixed dose combination¹⁵⁻¹⁷. Though these methods already exist but none of the methods reported simultaneous quantitative estimation of QUINA and HCTZ by diffuse reflectance using Fourier transform infrared spectrophotometry (DRIFTS), dual wavelength method using UV and stability indicating assay method using HPLC. Hence attempt was made towards development of assay method for simultaneous estimation of QUINA and HCTZ using DRIFTS, UV and HPLC. Green approach is used in the method developed by using DRIFTS, which is solvent free method of analysis. UV method was found to be faster. Method developed using HPLC was stability indicating.

EXPERIMENTAL

Quinapril hydrochloride (QUINA) and hydrochlorothiazide (HCTZ) were obtained as gift samples from Aurobindo Pharmaceuticals Ltd., Hyderabad and Macleods Pharmaceuticals Ltd., Mumbai respectively. Potassium bromide (IR grade) was purchased from Qualigenes Fine Chemicals (Mumbai, India). Analytical reagent (AR) grade sodium hydroxide and hydro-

Fig. 1. Structure of (a) quinapril hydrochloride (QUINA) and (b) hydrochlorothiazide (HCTZ)

chloric acid were purchased from Qualigens Fine Chemicals (Mumbai, India). Formic acid, hydrogen peroxide (H_2O_2) were purchased from S.D. Fine-Chem Ltd. (Mumbai, India). HPLC grade acetonitrile and methanol were purchased from S.D. Fine-Chem Ltd. (Mumbai, India). HPLC grade double distilled water was produced using quartz double distillation assembly from Lab-Sil Instruments (Bangalore, India). Marketed formulation (Acupil-H, Macleods Pharmaceutical Ltd., Mumbai) was purchased from local drug store.

FT-IR spectra were recorded by using Jasco 4100 FT-IR spectrophotometer DRIFTS system. UV spectra were recorded by using Jasco V-630 UV-visible double beam spectrophotometer. The output signals of FT-IR and UV spectrophotometer were monitored by using Spectra Manager software.

Chromatographic work was performed on Shimadzu Prominence binary gradient system (LC 20 AD) fitted with 20 μL injection loop and photo diode array detector (PDA) (SPD M20A). The output signals were monitored and processed using LC solution multi PDA software.

Degradation studies were carried out on water bath (Meta-Lab, Mumbai) equipped with thermostat for temperature control. A hot air oven (Scientico, Mumbai) was used to carry

out solid state thermal stress studies. Photostability studies were carried out in photostability chamber (Thermolab Scientific Equipments Pvt. Ltd., India). The photostability chamber consists of two ultraviolet (UV) and four white fluorescent lamps; both of these lamps were kept on during the photostability study. Calibrated lux and UV meters were used to measure visible illumination and near UV energy respectively. The data was recorded and processed using Stability V7.2T software on Dell computer. The separation was carried out on C_{18} HIQ-sil (150×4.6 mm, particle size $5 \mu\text{m}$) column at flow rate of 1 mL min^{-1} and wavelength maximum 215 nm. The mobile phase was composed of 0.01 M KH_2PO_4 buffer pH 3.5 (adjusted with orthophosphoric acid): methanol which was run in gradient mode as outlined in Table-1. The mobile phase in the ratio (50:50 v/v) was used for diluting the samples.

The pH of mobile phase was adjusted by using pH meter (Controlled Dynamics, India). Other equipments used were sonicator (Spectralab UCB 30) and analytical balance (Precissa XR 205 SMDR).

TABLE-1
GRADIENT ELUTION PROGRAMME

Time (min)	Buffer (KH_2PO_4 , pH = 3.5) (%)	Methanol (%)
0.01	43	57
20	23	77
30	43	57

DRIFTS method

Calibration curve method: Calibration curve method was used for quantitative analysis of QUINA and HCTZ. In this technique FT-IR spectrum of the drug was recorded in the range of $4000\text{--}400 \text{ cm}^{-1}$ and corresponding area of the absorption peak was measured with the help of software program. The absorption peak selected for one particular drug was sufficiently different than that of another drug to avoid interference. The overlain spectra of QUINA and HCTZ is shown in Fig. 2. The quantitative determination of QUINA and HCTZ were carried out by selecting peak area at 1680--

Fig. 2. Overlain FT-IR spectra for QUINA and HCTZ

1615 cm^{-1} (C=O stretch) and 1338-1284 cm^{-1} (O=S=O stretch), respectively.

Method validation

Linearity: The linearity of QUINA and HCTZ was determined at six concentration levels of 10-30 mg/50 mg Kbr for both the drugs. The samples were prepared in triplicate. The peak area *versus* concentration data was processed by least-square linear regression analysis

Precision: The intra-day and inter-day precision was carried out by analyzing both the drugs (10, 14 and 18 mg /50 mg Kbr) drug mixtures were prepared in triplicate on same day and on consecutive days.

Accuracy: The accuracy of the method was determined by conducting recovery studies of pure drug by standard addition method. It was carried out by spiking mixture of drugs (10 mg/50 mg Kbr) with three known concentrations of both drug (8, 10 and 12 mg/50 mg Kbr) in triplicate and then determining per cent recovery of added drug.

Assay of marketed formulation: Twenty tablets containing QUINA (10 mg) and HCTZ (12.5 mg) were weighed. Average weight of the tablet was calculated. The powder (252 mg) equivalent to 10 mg of QUINA and 12.5 mg of HCTZ was weighed. To weighed quantity of powder, IR grade potassium bromide was added up to 50 mg. This mixture was triturated in agate mortar and pestle and was placed in cup of diffuse reflectance assembly. After recording spectrum, peak areas of selected peak regions were measured to determine concentration of both the drugs.

Spectrophotometric method: Dual wavelength method¹⁸ was used to calculate the unknown concentration of a component of interest from multicomponent formulation. In this method two such wavelengths are selected where, absorbance difference for one of the component is directly proportional to its concentration while absorbance difference for another component equals to zero thus interference due to other component is avoided. For estimation of QUINA two wavelengths (220 and 230 nm) were selected where QUINA shows significant difference in absorbance while HCTZ shows same absorbance, hence absorbance difference of HCTZ is zero. This condition will avoid interference due to HCTZ in the estimation of QUINA. For estimation of HCTZ, 261 nm wavelength was selected where QUINA will not show any interference due to its zero absorbance at this wavelength. The overlain spectra of QUINA and HCTZ is shown in Fig. 3.

Fig. 3. Overlain spectra of QUINA and HCTZ (15 $\mu\text{g/mL}$ each)

The concentration of individual drug was determined by using following equation

For QUINA $y = 0.038x + 0.263$ (y = absorbance difference at 220 and 230 nm, x = concentration in $\mu\text{g/mL}$)

For HCTZ $y = 0.043x + 0.010$ (y = absorbance at 261nm, x = concentration in $\mu\text{g/mL}$)

Method validation: The developed method was validated as per ICH guideline Q2 (R1)¹⁹.

Linearity: Linearity of QUINA was determined by plotting absorbance difference values at 220 and 230 nm *vs.* concentration. Whereas linearity of HCTZ was determined at 261 nm wavelength. The linearity test solutions for QUINA (10-50 $\mu\text{g mL}^{-1}$) and HCTZ (5 to 25 $\mu\text{g mL}^{-1}$) were prepared using methanol at five concentration levels from the stock solution of QUINA (1000 $\mu\text{g mL}^{-1}$) and HCTZ (1000 $\mu\text{g mL}^{-1}$), respectively. The solutions were prepared in triplicate. The absorbance *versus* concentration data was processed by least-square linear regression analysis.

Precision: The intra-day and inter-day precision was carried out by analyzing QUINA (20,30,40 $\mu\text{g mL}^{-1}$) and HCTZ (10, 15, 20 $\mu\text{g mL}^{-1}$). Drug solutions were prepared in triplicate on same day and on consecutive days.

Accuracy: The accuracy of the method was determined by conducting recovery studies of pure drug by standard addition method. It was carried out by spiking mixture of drugs (QUINA 10 $\mu\text{g mL}^{-1}$ and HCTZ 10 $\mu\text{g mL}^{-1}$) with three known concentrations of standard drugs QUINA (8,10 and 12 $\mu\text{g mL}^{-1}$) and HCTZ (8, 10 and 12 $\mu\text{g mL}^{-1}$). Drug solutions were prepared in triplicate and per cent recovery of added drug was determined.

Assay of marketed formulation: Twenty tablets were weighed and crushed to obtain a fine powder. Tablet powder equivalent to 10 mg of QUINA and 12.5 mg of HCTZ was transferred to 25 mL volumetric flask. To it 15 mL of methanol was added and sonicated for 15 min. The volume was made up to the mark by using methanol. The resulting solution was filtered through Whatman filter paper no. 42 and filtrate was appropriately diluted with methanol to get concentration of 10 $\mu\text{g/mL}$ of QUINA and 12.5 $\mu\text{g/mL}$ of HCTZ. The absorbance was measured at the selected wavelengths and concentrations were determined. The analysis was carried out in triplicate.

RP-HPLC method

Stability indicating method: Stability indicating assay methods (SIAMs) can be specific or selective. Specific stability indicating assay method is developed to measure the active ingredient in the presence of all degradation products, excipients and additives expected to be present in the formulation. Selective stability indicating method is developed to measure active ingredients as well as degradation products in presence of excipients and additives²⁰.

Regulatory ICH guidelines Q1AR2²¹, Q3BR2²², Q6A²³ and FDA 21 CFR section 211²⁴ explicitly indicate the need of stress testing, identification of degradation products and development of validated stability indicating assay method. However these guidelines do not provide any specific practical methodology for stress testing²⁵. Hence, degradation conditions, concentration of degrading agent and time of degradation

are to be determined empirically to achieve 10 to 15 % of degradation of active ingredient.

Stress studies: Stress studies were carried out on each drug (QUINA and HCTZ) under variety of stress conditions *i.e.* acidic, alkaline and neutral hydrolysis, oxidation, heat and photolysis. Hydrolytic decomposition of drugs was carried out separately under acidic, alkaline and neutral condition by heating 1 mL each of HCl (1 N), NaOH (1 N) and water with 1 mL of 1000 mg mL⁻¹ drug at 80 °C for different time intervals. Samples were neutralized after required exposure. Samples treated with acid were neutralized by using equal strength of base and *vice versa*. Each drug solution, 1 mL of 1000 mg mL⁻¹ was treated with 1 mL of 15 % H₂O₂ at 80 °C for different time intervals for oxidative stress. Thermal and photo degradation was carried out using solid drug. In case of thermal degradation, the solid drug was heated in an oven within sealed glass ampoule at 70 °C for a period of three days. In the same way the control sample was maintained outside of the oven at room temperature. During photo degradation solid drug was exposed to 1.25 million lux hours of fluorescent light and 200 Watt hours/square meter of UV light along with control sample, which was covered with aluminum foil. Samples after required exposure under different stress conditions were diluted with the help of diluent to predetermined concentration of original drug (100 µg mL⁻¹) and injected in HPLC for analysis.

Preparation of samples for HPLC: The samples were diluted up to 10 mL with diluent in case of acid, base, neutral and oxidative stress to produce concentration of 100 µg mL⁻¹ of original drug. The solid samples which were exposed for photo and thermal stress were also weighed appropriately and diluted with suitable quantity of diluent to produce 100 µg mL⁻¹ of original drug.

Development of stability indicating assay (SIAM) method: Initial method development was carried out by using reverse phase chromatographic system to monitor degradation behaviour of individual drug (QUINA and HCTZ). HiQsil C18 (150 × 4.6 mm, 5 µm) column was used as stationary phase. The pK_a values of QUINA and HCTZ are 3.7, 5.2 and 7.9, respectively. It was observed that at pH 3.5, QUINA and HCTZ showed desirable retention behaviour in reversed phase chromatographic system. Under different stress conditions QUINA and HCTZ generated polar as well as non-polar

degradants. Initially HPLC analysis was performed on all stressed samples individually and then on mixture of all stressed samples. Gradient elution system was employed; different modifications in gradient program were carried out to obtain better resolution among the degradants and between drug and degradants. The best separation was achieved by using a mobile phase composed of 0.01 M KH₂PO₄ buffer pH 3.5 (adjusted with orthophosphoric acid): methanol which was run in gradient mode as outlined in Table-1. The flow rate was 1 mL/min and detection wavelength 215 nm.

Method validation: The developed method was validated as per ICH guideline Q2 (R1)¹⁹.

Linearity: Linearity of QUINA and HCTZ was determined by plotting peak area *versus* concentration. The linearity test solutions for QUINA (2-10 µg mL⁻¹) and HCTZ (2.5 to 12.5 µg mL⁻¹) were prepared from the stock solution of QUINA (1000 µg mL⁻¹) and HCTZ (1000 µg mL⁻¹) respectively. The solutions were diluted with the help of diluents (buffer: methanol, 50:50 v/v). The solutions were prepared in triplicate at five different concentration levels. The peak area *versus* concentration data was processed by least-square linear regression analysis.

Precision: The intra-day and inter-day precision was carried out by analyzing QUINA (4, 6, 8 µg mL⁻¹) and HCTZ (5, 7.5, 10 µg mL⁻¹). Drug solutions were prepared in triplicate on same day and on consecutive days.

Accuracy: The accuracy of the method was determined by conducting recovery studies of pure drug by standard addition method. It was carried out by spiking mixture of drugs (QUINA 4 µg mL⁻¹ and HCTZ 5 µg mL⁻¹) with three known concentrations of standard drugs QUINA (3.2, 4 and 4.8 µg mL⁻¹) and HCTZ (4, 5 and 6 µg mL⁻¹). Drug solutions were prepared in triplicate and percent recovery of added drug was determined.

Assay of marketed formulation: Twenty tablets were weighed and crushed to obtain a fine powder. Tablet powder equivalent to 10 mg of QUINA and 12.5 mg of HCTZ was transferred to 25 mL volumetric flask. To it 15 mL of methanol was added and sonicated for 15 min. The volume was made up to the mark by using methanol. The resulting solution was filtered through membrane filter (0.45 µ). The Filtrate was diluted with the help of diluent to get concentration of 10 µg/mL

Fig. 4. HPLC Chromatogram of HCTZ and QUINA with their degradation products

DRIFTS-calibration curve method: The developed calibration curve method was validated as per ICH guidelines (Table-2) and successfully used for analysis of QUINA and HCTZ in marketed formulation (Table-3). The FT-IR spectrum of commercial tablet sample containing QUINA and HCTZ showed that the excipients do not show interference with drug peaks.

Spectrophotometric-dual wavelength method: The developed dual wavelength method was validated as per ICH guidelines (Table-2) and successfully used for analysis of QUINA and HCTZ in marketed formulation (Table-3). The UV spectrum of commercial tablet sample containing QUINA and HCTZ also confirm that the excipients do not show interference with drug absorption.

RP-HPLC-stability indicating assay method: The stress studies were carried out on both the drugs (QUINA and HCTZ). Both the drugs were subjected to hydrolysis (acidic, alkaline

and neutral), oxidation, thermal stress and photolysis. The stress conditions were optimized to obtain degradation products after 15-20 % degradation of both the drugs (QUINA and HCTZ). The degradation products obtained under all the stress conditions were separated by using HPLC. The degradation products generated under different stress conditions carry the notations from I to V in accordance with the sequence in which peak appears from left to right on the HPLC chromatogram (Fig. 4). The results of the stress study are given in following Table-4.

The developed method was validated as per ICH guideline Q2 [R1]. The summary of the validation parameter is given in Table-2. The method was applied for analysis of marketed formulation. The assay results are given in Table-3.

The present research paper describes application of UV spectrophotometric method, FT-IR spectrophotometric method and HPLC method for determination of QUINA and HCTZ in fixed dose marketed formulation. The proposed methods are simple, specific, accurate and precise. The analyses of marketed formulation do not show any interference of additives and

Sr. No.	Parameter	UV Spectrophotometric method		IR Spectrophotometric method		RP-HPLC method	
		QUINA	HCTZ	QUINA	HCTZ	QUINA	HCTZ
1.0	Linearity						
1.1	Equation of line	y = 0.043x + 0.010	y = 0.038x + 0.263	y = 443.7x + 18274	y = 454.3x + 10582	y = 10085x + 10662	y = 35608x + 4995
1.2	r ²	0.999	0.990	0.997	0.990	0.995	0.997
2.0	Range	10-50 µg/mL	5.0-25 µg/mL	10-30 mg/50 mg KBr	10-30 mg/50 mg KBr	2.0-10.0 µg/mL	2.5-12.5 µg/mL
3.0	LOD	1.15 µg/mL	1.21 µg/mL	0.09 mg/50 mg KBr	0.07 mg/50 mg KBr	0.60 µg/mL	0.54 µg/mL
4.0	LOQ	3.48 µg/mL	3.68 µg/mL	0.30 mg/50 mg/ KBr	0.22 mg/50 mg KBr	1.83 µg/mL	1.65 µg/mL
5.0	Precision	Method	Actual conc. (µg/mL)	Intra-Day Measured Conc. (µg/mL) Mean ± RSD (%)	Intra-Day Measured Conc. (µg/mL) Mean ± RSD (%)	Inter-Day Measured Conc. (µg/mL) Mean ± RSD (%)	Inter-Day Measured Conc. (µg/mL) Mean ± RSD (%)
		UV Spectrophotometric method	20 10 30 15 40 20	19.91 ± 1.31 30.12 ± 0.94 39.66 ± 0.32	9.93 ± 1.84 14.96 ± 1.36 20.07 ± 1.09	19.85 ± 1.59 30.45 ± 1.12 39.45 ± 1.19	9.88 ± 1.69 14.80 ± 1.86 19.90 ± 1.67
		FT-IR Spectrophotometric method	10 10 14 14 18 18	9.91 ± 0.18 14.09 ± 0.73 18.15 ± 0.56	9.87 ± 1.54 14.25 ± 1.39 17.89 ± 1.32	10.15 ± 0.91 13.94 ± 0.91 17.93 ± 0.98	10.19 ± 1.89 13.80 ± 1.23 18.30 ± 1.45
		RP-HPLC method	4 5 6 7.5 8 10	4.08 ± 1.72 5.95 ± 1.78 7.91 ± 1.77	4.99 ± 1.81 7.45 ± 1.21 9.97 ± 1.41	3.91 ± 1.99 6.08 ± 1.10 8.07 ± 1.27	5.06 ± 1.29 7.40 ± 0.80 9.92 ± 1.25
6.0	Accuracy		Spiked conc. (µg/mL)	Measured Conc. (µg/mL) Mean ± RSD (%)	Recovery (%)	Measured Conc. (µg/mL) Mean ± RSD (%)	Recovery (%)
		UV Spectrophotometric method	8 8 10 10 12 12	8.05 ± 1.14 9.94 ± 0.80 11.92 ± 0.50	100.71 99.41 99.40	8.00 ± 1.31 10.07 ± 0.29 12.02 ± 0.29	100.1 100.76 100.2
		FT-IR Spectrophotometric method	8 8 10 10 12 12	7.93 ± 1.10 10.03 ± 0.97 11.99 ± 0.78	99.12 100.03 99.91	7.99 ± 0.72 10.10 ± 0.88 12.16 ± 0.41	99.87 100.10 101.33
		RP-HPLC method	3.2 4 4 5 4.8 6	3.21 ± 1.13 4.00 ± 1.83 4.81 ± 1.00	100.33 100.01 100.41	3.96 ± 0.9510 5.01 ± 0.4654 6.02 ± 1.0236	99.07 100.35 100.49

TABLE-3
RESULTS OF ANALYSIS OF TABLET FORMULATION

Tablet formulation	Label claim/(mg per tablet)	Amount of drug estimated	% of label claim Mean $\pm$ R. S. D. (%)
Acupil-H (Pfizer Limited, Mumbai)	HCTZ- UV	12.50	100.00 $\pm$ 0.78
	IR	12.44	99.56 $\pm$ 1.09
	HPLC	12.45	99.66 $\pm$ 0.58
	QUINA- UV	9.87	98.78 $\pm$ 0.90
	IR	10.04	100.42 $\pm$ 0.81
	HPLC	10.71	100.71 $\pm$ 1.01
Number of tablets = 20, n = 3			

TABLE-4
SUMMARY OF DEGRADATION BEHAVIOUR OF HCTZ AND QUINA

Sr. No.	Name of drug	Stress condition	Notation in chromatogram
1	Quinapril hydrochloride	Acid hydrolysis (1N HCl) 80 °C, 2.0 h	II and III
		Base hydrolysis (1N NaOH) 80 °C, 2.0 h	IV and V
		Neutral hydrolysis 80 °C, 2.0 h	-
		Oxidative stress (15 % H ₂ O ₂)	-
		Thermal stress 70 °C, 3.0 days	-
		Photolysis	-
2	Hydrochlorothiazide	Acid hydrolysis (1N HCl) 80 °C, 2.0 h	I
		Base hydrolysis (1N NaOH) 80 °C, 2.0 h	I
		Neutral hydrolysis 80 °C, 2.0 h	I
		Oxidative stress (15 % H ₂ O ₂)	I
		Thermal stress 70 °C, 3.0 days	-
		Photolysis	-
- No degradants were observed			

excipients. FT-IR analysis is green analytical technique where the analysis is carried out without use of organic solvents.

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