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Ultra Performance Liquid Chromatography Assay for Cinnarizine in Lipid-Based Formulations

M. ABDEL-HAMID, A. SHAHBA, K. MOHSIN* and F. ALANAZI

Kayyali Chair for Pharmaceutical Industries, Department of Pharmaceutics, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia

*Corresponding author: Fax: +966 1 4676295; Tel: +966 1 4676228; E-mail: mkazi@ksu.edu.sa

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The objective of present study is to evaluate UV-VIS spectrophotometry and ultra performance liquid chromatography (UPLC) methods for quantification of cinnarizine in lipid-based formulations. The direct spectrophotometric method was carried out at 253 nm, which gave good linearity (R^2 = 0.999), but was unsuitable for cinnarizine quantification within lipid-based formulation due to significant matrix interference. While the ultra performance liquid chromatography reversed phase method was composed of isocratic mobile phase, 0.25 % trifluoroacetic acid in 50 % acetonitrile with flow rate 0.5 mL/min, column BEH C_{18} (2.1 × 50 mm, 1.7 µm) found to be rapid (1min run), selective with well resoluted cinnarizine peak from different lipid matrices and degradation product (resolution >2.0) and sensitive (LOD = 0.1ppm and LLOQ = 0.5ppm) at 251 nm. The accuracy and precision were within the standard FDA limits. The study suggests that the developed ultra performance liquid chromatography method can be used for the assessment of drug purity, stability, lipid-formulation release profile and dissolution rate without any interference of excipients and/or degradation products.

Key Words: Ultra performance liquid chromatography, UV-VIS spectrophotometry, Cinnarizine, Lipid-based formulation, Stability-indicating assay.

INTRODUCTION

Cinnarizine, [(E)-1-(diphenylmethyl)-4-(3-phenyl-2-propenyl) piperazine] (Fig. 1), is used as an antihistaminic drug, calcium entry blocker for the treatment of cerebral and peripheral vascular insufficiency. It is also prescribed for the control of nausea and vomiting, vestibular symptoms and gives accurate efficiency for different syndromes such as meniere's disease or cerebrovascular vertigo[1]. After oral administration, the drug is rapidly absorbed with maximum plasma concentration within 1-3 h and provides elimination half-life of about 3-4 h[2].

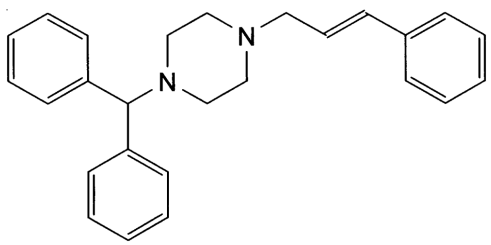


Fig.1. Chemical structure of cinnarizine

The determination of cinnarizine was carried out by several analytical methods using derivative spectrophotometry[3], high performance liquid chromatography with ultraviolet detection (UV)[4], high performance liquid chromatography with fluorometric detection[5] and gas chromatography[6]. However, these methods often suffered from several disadvantages including a sophisticated sample preparation, long running time, a complex and time-consuming analytical procedure, use of toxic reagents for extraction and/or derivatization either pre-column or post-column which increased the complexity of chromatograms[1,7].

Single-form determination of cinnarizine can be achieved by direct spectrophotomery through measuring the drug at 253 nm in 0.1M HCl[8]. This method sounds to be attractive for the *in-vitro* determination because it is rapid, simple, cost-effective and provides satisfactory results within the optimum concentration ranges. None the less, within the scope of the current studies it was not suitable to determine cinnarizine in lipid-based formulations due to significant interference between cinnarizine and the formulation excipients over the working wavelength range as thoroughly discussed in present studies.

Additionally, cinnarizine presents a poor chemical stability and shows extremely rapid degradation in aqueous solutions at pH 3.12 -7.32[9]. So, there was urging need for the development of an analytical method that is able to specifically determine cinnarizine in presence of its degradation products as well as formulation excipients.

In present studies, a specific, rapid and simple isocratic reverse phase chromatographic method employing ultra performance liquid chromatography was developed for the quantification of cinnarizine in pure and blended pharmaceutical grade lipid-based formulations. Ultra performance liquid chromatography is relatively new technique giving new possibilities in liquid chromatography, especially concerning decrease of time and solvent consumption. Ultra performance liquid chromatography system is designed in a special way to withstand high system back-pressures without causing any negative influence on analytical column[10]. The proposed method was successfully applied to the analysis of lipid-based formulations containing cinnarizine with no interference from dosage form excipients or cinnarizine degradation products. The method was validated with respect to the standard FDA guidelines.

EXPERIMENTAL

Cinnarizine (purity > 99.5) was generously donated by FDC Limited, Maharashtra, India. High performance liquid chromatography-grade acetonitrile and hydrochloric acid were obtained from BDH Laboratory Supplies (BDH Chemicals Ltd., Poole, U.K.). Trifluroacetic acid was purchased from Winlab (Gemini-house, UK). High purity water was obtained through a Milli-Q Integral Water Purification System (Millipore, Bedford, MA). All other reagents were of analytical grade and were used without further purification.

UV spectrophotometry: The UV system consisted of a labomed UV-visible spectro-photometer (model UVD-3200) with 10 mm quartz cuvettes connected to a computer system fitted with RS-232 PC interface for windows (Labomed, Inc. USA). The detector wavelength was set at 253 nm[8].

A stock solution of cinnarizine was prepared by dissolving 100 mg cinnarizine in 200 mL 0.1M HCl, resulting in a solution containing 500 ppm cinnarizine. The working standard solutions were prepared by suitable dilution of the stock solution with 0.1 M HCl as required.

12 serial standard solutions ranging from 1.0-40.0 ppm were prepared by suitable dilution of the stock or working solution with 0.1 M HCl. Calibration curves were obtained by plotting absorbance against drug concentrations and regression equations were computed.

Formulation matrix effect: Direct spectrophotometric methods often suffer the big disadvantages of their low selectivity and accuracy due to the expected spectral overlapping between the analyte and formulation excipients[11]. Thus, UV spectral scanning of both drug-containing and drug-free formulations should be always performed at first to detect any possible interference. In this study, spectral scanning of both standard cinnarizine solution and drug-free lipid formulation (diluted in the same solvent) was performed in the wavelength range of 200-300 nm.

Ultra-performance liquid chromatography: Chromatographic separation was optimized with respect to the stationary and mobile phase compositions, flow-rate, sample volume and detection wavelength. The ultra performance liquid chromatography system consisted of an Acquity® ultra performance liquid chromatography binary solvent manager equipped with an Acquity® automatic sample manager and an Acquity® ultra performance liquid chromatography photodiode array (PDA) eλ detector obtained from Waters (Waters Inc., Bedford, MA, USA). Separation was achieved by reverse-phase isocratic elution using a mobile phase consisting of 0.5 % trifluroacetic acid : acentonitrile (50:50) delivered at a flow rate of 0.5 mL/min through an Acquity® ultra performance liquid chromatography BEH C_{18} column (2.1 × 50 mm, 1.7 µm) kept at 50 ºC. The run time was 1 min. Freshly prepared mobile phase was filtered through an online 0.2 µm filter and degassed continuously by an online degasser within the ultra performance liquid chromatography system. The detector wavelength was set at 251 nm and the injection volume was 1.0 µl.

Stock solutions of cinnarizine were prepared by dissolving 125 mg cinnarizine in 250 mL acetonitrile, resulting in a solution containing 500 ppm cinnarizine. The working standard solution was prepared by diluting 1 mL of stock solution into a 50 mL volumetric flask with acetonitrile to give a 10 ppm cinnarizine concentration. Serial standards of cinnarizine were prepared to cover the following concentration range (0.1-200.0) ppm. Calibration curves were obtained by plotting peak area against standard drug concentration and regression equations were computed.

Validation of bioanalytical method: The aforementioned method was validated in terms of linearity, specificity, precision and accuracy, limit of detection and lower limit of quantification according to the FDA guidelines of bioanalytical method validation[12,13].

Linearity and range: Appropriate volumes of cinnarizine stock solution (500 ppm) or working standard solution (10 ppm) were utilized to prepare 11 non-zero standard drug concentrations covering the calibration range from (0.1-200 ppm). Four quality control samples with the selected concentrations (0.7, 36.4, 140.8, 184.5 ppm) were prepared to cover the desired range. Quality control samples were prepared by spiking the lipid-formulation with known amount of cinnarizine, then diluting the mixture with appropriate volume of acetonitrile.

Each solution was injected at 6 replicates on three consecutive days for validation. Calibration solutions were injected in ascending order in each validation run and the other samples were distributed randomly through the run.

Linear regression equation and correlation coefficient (R^2) were employed to statistically evaluate the linearity of the results[14,15].

Specificity: Specificity of the method was needed to be assessed to evaluate the matrix influence between the drug and different lipid-based formulations as well as to ensure the validity of the method to be further utilized as a stability-indicating assay.

To evaluate the specificity of the method, drug free quality control zero samples were carried out through the assay procedure and the retention times of the lipid formulation components were compared with that of cinnarizine analyte[14]. The specificity of the method towards the intact drug was also studied by determination of the resolution (R) between the drug peak and the nearest degradation product[16].

Accuracy and precision: Intra-day accuracy and precision evaluations were performed through six replicates determinations of 11 cinnarizine standards within the same day. Inter-day

accuracy and precision were assessed by six replicates analysis of the following: lower limit of quantification, low, medium and high quality control samples on three consecutive days. The overall precision of the method was expressed as relative standard deviation and accuracy of the method was expressed in term of % drug recovered[14].

Limit of detection and lower limit of quantification: The limit of detection and lower limit of quantification were determined by serial dilutions of cinnarizine stock solutions in order to obtain signal to noise (S/N) ratio of at least≈3:1 for limit of detection and ≈10:1 for lower limit of quantification[12].

RESULTS AND DISCUSSION

UV spectrophotometry: The absorption spectrum of cinnarizine in aqueous acid (0.1 M HCl) (Fig. 2) displays a well-defined absorption peak at 253 nm, which is in agreement with the reported methods[8]. There was not any significant absorption observed by the drug-free aqueous acid at this wavelength.

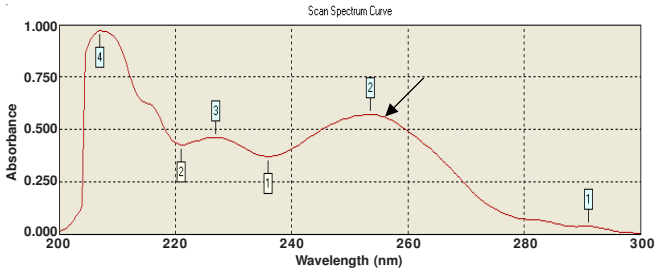


Fig. 2. UV absorbance spectrum of cinnarizine in 0.1M HCl (conc. = 10 ppm). The arrow shows cinnarizine peak at 253 nm.

Under the optimum experimental conditions, Beer Lambert law was valid over the concentration range of 4-35 ppm (Table-1, Fig. 3). Least-squares analysis of the calibration data gives the relationship:

$$y = (0.05669)\ x - (0.02847),\ R^2 = 0.999$$

where y denotes the absorbance of cinnarizine at 253 nm, x: the cinnarizine concentration (ppm) and R^2: the correlation coefficient.

TABLE-1
DATA OF BACK-CALCULATED CINNARIZINE CONCENTRATIONS OF THE CALIBRATION STANDARDS IN AQUEOUS ACID (0.1M HCl) BY UV-SPECTROPHOTOMETRY

ID	Nominal conc. (ppm)	Absorbance	Back calculated conc. (ppm)	Accuracy %
Standard 1	1	0.0147	0.76	76.15
Standard 2	2	0.0717	1.77	88.35
Standard 3	3	0.1324	2.84	94.59
Standard 4	4	0.1891	3.84	95.95
Standard 5	5	0.2536	4.98	99.51
Standard 6	10	0.5544	10.28	102.82
Standard 7	15	0.8302	15.15	100.98
Standard 8	20	1.1297	20.43	102.15
Standard 9	25	1.4313	25.75	103.00
Standard 10	30	1.7129	30.72	102.39
Standard 11	35	1.9809	35.44	101.27
Standard 12	40	2.1433	38.31	95.77

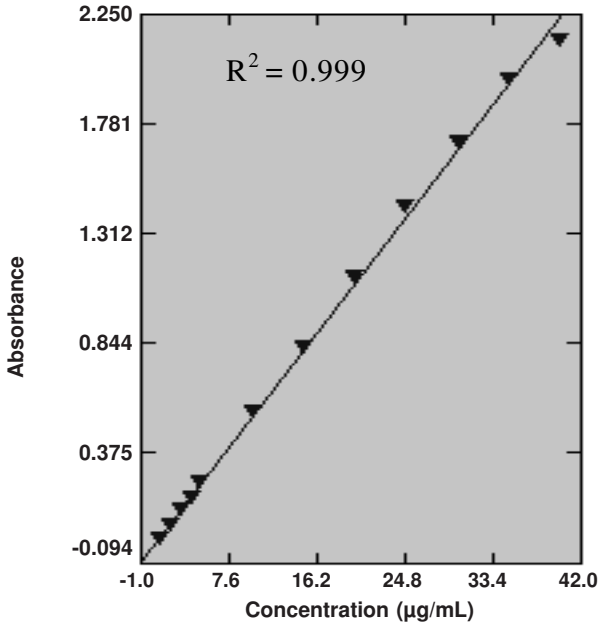


Fig. 3. Calibration curve of cinnarizine in aqueous acid (0.1M HCl) by UV-spectrophotometric method.

However, spectral scanning of both standard cinnarizine solution and drug-free lipid formulation (diluted in the same aqueous acid solvent) revealed significant spectral overlapping within the working wavelength range (Fig. 4) turning the direct spectrophotometric method invalid for determination of cinnarizine within lipid-based formulations.

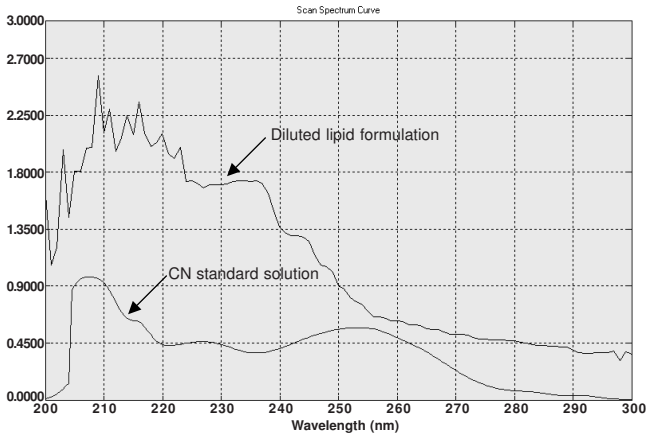


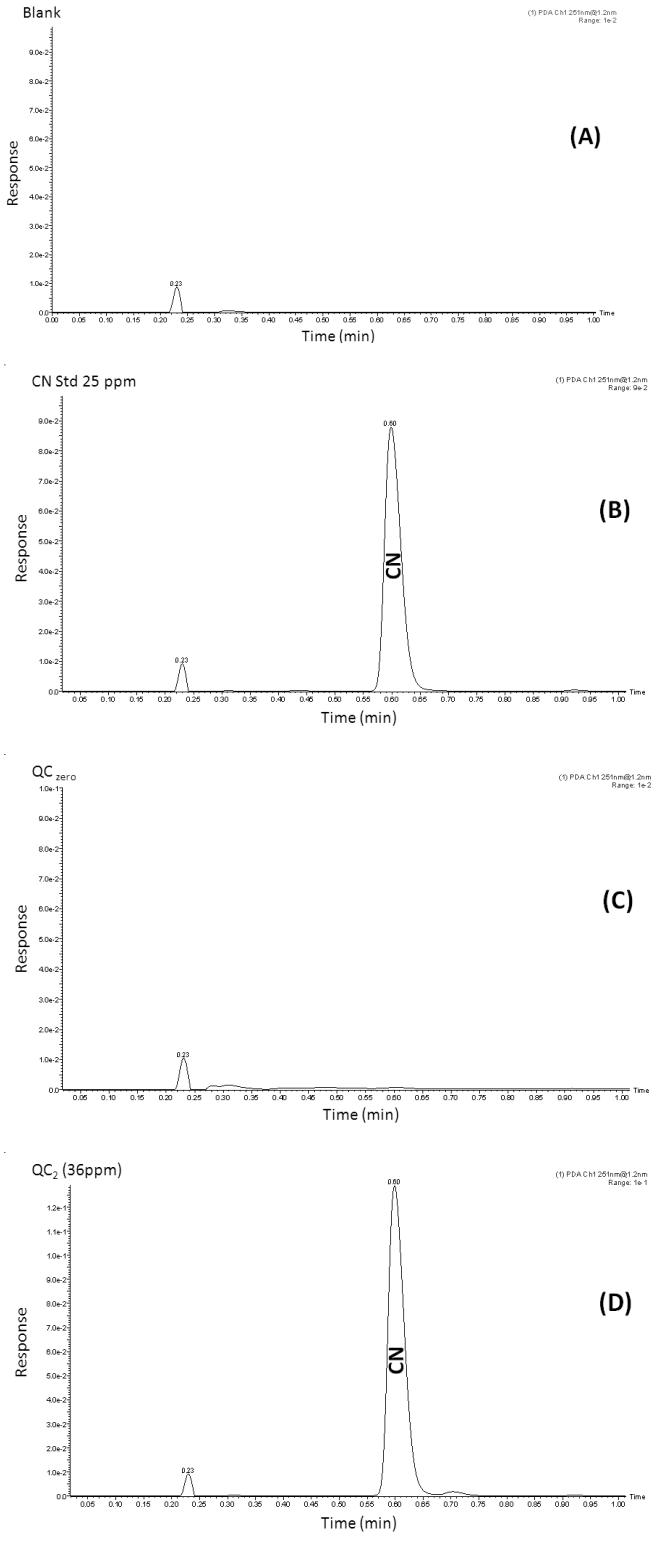
Fig. 4. UV absorbance spectrum of cinnarizine 10 ppm (lower curve) and drug-free lipid formulation (upper curve) diluted in the same solvent

Ultra-performance liquid chromatography

Separation: Fig. 5 shows the representative chromatograms of blank sample (a), standard solution of cinnarizine (b), drug-free lipid formulation (quality control zero) sample (c), drug-containing lipid formulation quality control sample (d) and degraded cinnarizine sample (e).

Separation and detection of cinnarizine without any interference were ideal by the ultra performance liquid chromatography assay. Initially, it was difficult to separate cinnarizine from its related and/or degraded substances by the UV analysis at 253 nm. This was caused by the lipid drug interference at

the targeted cinnarizine detection wavelength. However, the chromatographic result of ultra performance liquid chromatography technique shows that the sensitivity and selectivity of this procedure is good enough to determine cinnarizine within its degradation products. The cinnarizine analyte was well separated from the degradation products at retention time of *ca.* 0.61 min, while the main degradation products were eluted at retention times of *ca.* 0.72 and 0.91 min (Fig. 5e). The total run time was *ca.* 1.0 min. The peaks were of good shape, completely resolved one from another.



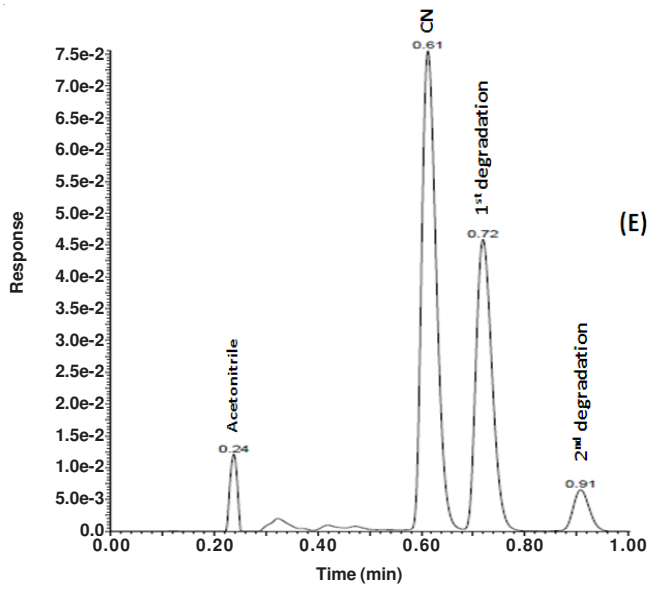


Fig. 5. Ultra performance liquid chromatography chromatograms of blank sample (a), standard solution of cinnarizine 25 ppm (b), drug-free lipid formulation (quality control zero) sample (c), drug-containing lipid formulation (quality control 2) sample (d) and degraded cinnarizine sample (e)

Validation of bioanalytical method

Linearity and range: The peak area response of cinnarizine was linear over the concentration range between 0.5 and 200 ppm (Fig. 6). The results of linear regression give the following mean equation:

$$y = 118.3457 (\pm 1.0073)\ x + 17.029 (\pm\ 0.5286)$$

where y denotes the peak area of the analyte and x denotes the concentration of the analyte. These results show excellent linearity over the interval studied with correlation coefficient (r) = 0.9991 (± 0.00008)[17].

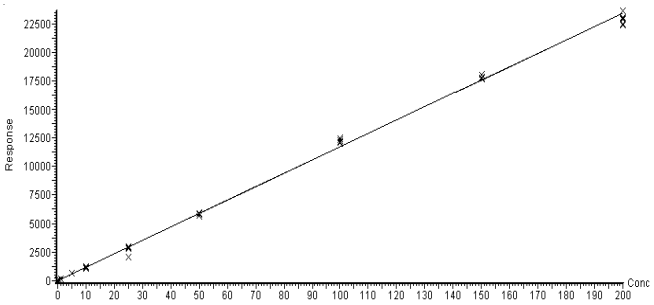


Fig. 6. Ultra performance liquid chromatography calibration curve of cinnarizine in acetonitrile.

Accuracy: The accuracy was calculated as the % of drug recovered after analysis relative to the corresponding nominal concentrations. The intra-day (Table-2) accuracy was between 96.87 % and 101.72 % and the inter-day (Table-3) was accuracy is between 83.36 % and 101.64 %. These values of the % drug recovered reflect the accuracy of the assay method and match with the acceptance criteria for FDA guidelines[13].

Precision: The results of intra-day and inter-day precision are presented in Tables 2 and 3, respectively. The developed method was found to be precise as the intra-day standard deviation (SD) values (Table-2) of six replicate analysis, at

TABLE-2
ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY DATA OF INTRA-DAY BACK-CALCULATED CINNARIZINE CONCENTRATIONS OF THE CALIBRATION STANDARDS IN ACETONITRILE.

Nominal conc. (ppm)	Back calculated concentrations (ppm)									
	1st	2nd	3rd	4th	5th	6th	Mean	S.D	Precision %	Accuracy %
0.5	0.49	0.49	0.51	0.50	0.52	0.49	0.50	0.01	2.06	100.07
1	1.00	1.02	1.03	1.03	1.01	1.02	1.02	0.01	1.14	101.72
5	4.97	5.10	5.04	5.03	4.95	4.96	5.01	0.06	1.20	100.14
10	10.06	10.01	10.00	9.82	9.72	10.03	9.94	0.14	1.36	99.41
25	24.21	24.28	24.24	24.57	24.09	23.91	24.22	0.22	0.90	96.87
50	50.64	50.17	49.61	51.05	49.94	49.75	50.19	0.55	1.10	100.38
100	100.00	99.80	99.57	101.37	99.95	99.95	100.11	0.64	0.64	100.11
150	150.64	148.09	146.61	148.94	148.03	147.55	148.31	1.37	0.93	98.87
200	201.27	203.28	199.80	206.15	199.72	202.91	202.19	2.45	1.21	101.09

the concentration range of 0.5-200 ppm, varied from 0.01 to 2.45 ppm with the coefficient of variation (CV; precision) ranging from 0.64 % to 2.06 %. Moreover, the inter-day (Table -3) SD values of six replicate determinations in three consecutive days were between 0.01 and 2.21 ppm with coefficient of variation being in the range of 0.98 to 1.48%. These perfectly low values of both standard deviation and coefficient of variation indicate the precision of this method[18].

TABLE-3
ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY DATA OF INTER-DAY ACCURACY AND PRECISION OF CINNARIZINE QUALITY CONTROL SAMPLES

Day of analysis	QC1	QC2	QC3	QC4
	0.7 ppm	36.4 ppm	140.8 ppm	184.5 ppm
1st day	0.60	35.72	144.00	182.63
	0.58	35.31	143.00	180.23
	0.60	35.58	142.39	180.93
	0.59	36.51	144.56	181.52
	0.60	35.85	140.08	179.35
	0.61	35.76	140.29	174.63
2nd day	0.60	35.95	143.02	181.46
	0.61	36.23	142.84	181.18
	0.58	36.02	142.28	179.79
	0.59	36.01	142.65	181.82
	0.60	36.41	141.90	179.29
	0.59	35.88	142.47	181.84
3rd day	0.59	36.81	145.19	184.57
	0.60	36.31	143.75	181.54
	0.59	36.53	144.42	182.02
	0.58	36.28	143.64	181.39
	0.60	36.45	144.35	182.19
	0.60	36.65	144.53	184.86
Mean	0.59	36.13	143.07	181.18
SD	0.01	0.40	1.41	2.21
Precision	1.48	1.11	0.98	1.22
Accuracy	83.36	99.18	101.64	98.18

Specificity: The specificity of the developed ultra performance liquid chromatography method for cinnarizine was investigated in order to obtain an indication of the possible interferences from the degradation product(s) of the drug under the studied stress conditions. The results in Fig. 5 show that there is no interference with cinnarizine peak. Cinnarizine is observed to be well resolved from the degradation peak (Fig. 5e). R values were calculated based on measuring peak width at half-height[19].

$$R = \frac{2(t_2 - t_1)}{1.70(W_{1,h/2} + W_{2,h/2})}$$

where R denotes the resolution, t_1 and t_2 are the retention times of two peaks and $W_{1,h/2}$ and $W_{2,h/2}$ represent the peak width at half-height of the two peaks.

Resolution value between cinnarizine and first degradation product was 2.16 and between the first and second degradation product was 3.4 (Fig. 5e). These results confirm that the developed ultra performance liquid chromatography method could be efficiently used as stability-indicating method for cinnarizine. In addition, there were no significant interfering peaks present in randomly selected drug free lipid formulation (quality control zero) samples at cinnarizine retention time (Fig. 5b, 5c).

Limit of detection and lower limit of quantification: The lower limit of quantification was 0.5 ppm, which estimated to be the lowest concentration of the standard curve that can be measured with acceptable accuracy and precision for the cinnarizine analyte and with S/N ratio of 80.4. On the other hand, limit of detection was 0.1 ppm with S/N ratio of 8.1.

Application: Within the scope of the current project, the developed ultra performance liquid chromatography method has been successfully used for the quantification of cinnarizine concentration in solubility, stability, dynamic dispersion and dissolution studies of lipid-based formulations. Fig. 7 shows an example of the dissolution profile of cinnarizine from an oral self-emulsifying lipid-based formulation in simulated gastric medium (pH 1.2). The lipid formulation consisted of mixture of long chain fatty acid, medium chain di- and mono-glycerides along with water-soluble surfactant and contained the standard dose (25 mg) of cinnarizine. The results showed that the method has capability to quantify as low as 5 % release of 25 mg cinnarizine formulation in 500 mL simulated gastric or intestinal media.

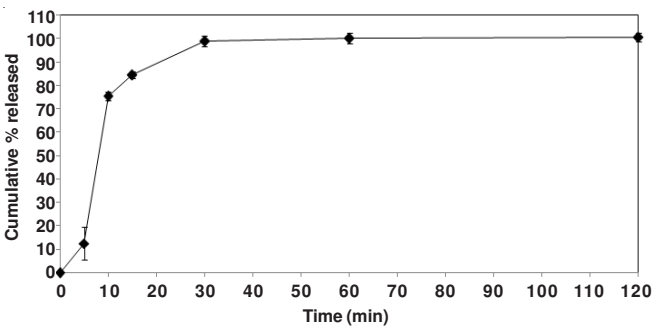


Fig. 7. Release profile of cinnarizine from a self-emulsifying lipid-based formulation in simulated gastric medium (pH 1.2). Data are expressed as mean ± S.E, n = 3

Conclusion

The developed ultra performance liquid chromatography method of analysis provides a reliable, reproducible and specific assay for cinnarizine in pure and pharmaceutical formulations. The described method is sensitive enough to detect as low as 0.1 ppm and exclusively offer a rapid determination of cinnarizine (1.0 min run time). No significant interferences were caused by the formulation excipients, diluents or degradation products.

The validation method allows quantification of cinnarizine in pure and pharmaceutical formulations in the range from 0.5 to 200 ppm. Compared to previously reported methods, the present assay method assessed extensive validation parameters as per FDA guideline. The method has shown acceptable precision, accuracy and adequate sensitivity and also demands to be used in further studies.

The established method satisfies the system suitability criteria, peak integrity and resolution between the parent drug and degradation products. The results clearly indicate that the current assay method is attractive due to the good selectivity and specificity for quantitative determination of cinnarizine in lipid-based formulation and also suitable for stability measurements and characterization of degradation products.

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