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Development of HPTLC method for the estimation of ondansetron hydrochloride in bulk drug and sublingual tablets

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A new, simple, rapid, accurate and precise high performance thin layer chromatography (HPTLC) method has been developed for the estimation of ondansetron hydrochloride in bulk and sublingual tablets. The mobile phase composition was chloroform : ethyl acetate : methanol : ammonia (9 : 5 : 4 : 0.1 *v*/*v*). Spectrodensitometric analysis of ondansetron was carried out at 254 nm and a symmetrical, well-resolved, well-defined peak was obtained at mean retardation factor (R_f) 0.52±0.02. The calibration plot was linear in the range 200-1200 ng/spot and showed good linear relationship with coefficient of regression, $R^2 = 0.9952$ with respect to peak area. The method was validated according to the guidelines of the International Conference on Harmonization (ICH Q2(R1). The limit of detection and quantitation were 14.83 and 44.92 ng per spot, respectively. The recovery study was carried out by standard addition method and the percentage recovery was found to be 99.34 ± 1.08. Therefore it was concluded that the proposed developed HPTLC method can be applied for identification and quantitative determination of ondansetron in bulk drug and dosage forms.

Keywords: ondansetron; high performance thin layer chromatography; method validation; Sublingual tablets; ICH

Introduction

Ondansetron is the first of several selective 5-hydroxytryptamine ($5\text{-}HT_3$) receptor antagonists available as an antiemetic agent. Its use in the settings of acute nausea and vomiting associated with highly and moderately emetogenic chemotherapy and radiotherapy to the upper abdomen are now well established.[1–3] Unlike metoclopramide, ondansetron does not block dopamine subtype-2 receptors, and therefore does not induce undesirable side effects, such as extrapyramidal reactions. Ondansetron hydrochloride has been used by oral and injectable administrations. It is rapidly absorbed orally, but extensively metabolized by the liver[4] and is usually administered 30 min before chemotherapy. Chemically, it is (±) 1, 2, 3, 9-tetrahydro-9-methyl-3-[(2-methyl-1H-imidazole-1-yl) methyl]-4H-carbazole-4-monohydrochloride dehydrate and official in USP/IP.[4–5]

Literature review revealed high performance liquid chromatography (HPLC) method[7–8] in human plasma, spectrophotometric method[9] and high performance thin layer chromatography (HPTLC) method[10] in combination solid dosage form, while no analytical method has been reported for the estimation of ondansetron in bulk drug and in single dosage forms. The HPLC technique is excellent with respect to selectivity and sensitivity, but it cannot be used for routine analysis because of its speciality requirement and cost. In view of this, HPTLC-based methods can be considered a good alternative, as it is a powerful analytical technique because of its reliability, simplicity, reproducibility, and speed.[11,12] A major advantage of HPTLC is its ability to analyze several samples simultaneously using a small quantity of mobile phase. This reduces time and cost of analysis.[13–15] Unlike HPLC, HPTLC has no limitation on the choice of mobile phase. Mobile phases having pH 8 and above can be employed.[16]

The main objective of the present work was to develop an economical, specific, accurate, reproducible HPTLC densitometric method for the estimation of ondansetron hydrochloride (OH) in bulk drug and in sublingual tablets pharmaceutical dosage forms developed in-house and validate it according to ICH guidelines Q2 (R1).

Materials and methods

Materials

Ondansetron hydrochloride reference standard was obtained as a gift sample from Brawn Lab. Ltd, (Faridabad, India). Analytical grade reagent chloroform, ethyl acetate, methanol, ammonia were supplied from Merck (Mumbai, India). All other reagents were of analytical grade.

HPTLC instrumentation

Solution of sample was applied to silica gel 60 F_{254} plates (10 cm × 10 cm, 0.2 mm thickness, E. Merck, Mumba, India) using a Camag Linomat V (Camag, Muttenz, Switzerland) equipped with a 100-µl

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Camag syringe. The samples were streaked in the form of bands of width 5 mm and a constant application rate of 100 nl/s was employed and space between two bands was 5.5 mm. The mobile phase consisted of chloroform : ethyl acetate: methanol : ammonia (9 : 5 : 4 : 0.1 *v*/*v*). Development of the plates were carried out in a twin-trough glass chamber (12.5 cm × 12.5 cm × 5 cm) saturated with the mobile phase for up to 30 min at room temperature (25 ± 2 °C) and a relative humidity of 55 ± 5%. The migration distance was 8 cm. Camag TLC scanner III with wincats software was used for densitometric scanning of the developed plates in the absorbance mode at 254 nm. The slit dimension was kept at 4 mm × 0.1 mm and 20 mm/s scanning speed was employed. The source of radiation utilized was deuterium lamp emitting continuous UV spectrum in the range of 190–400 nm. Evaluation was performed using linear regression analysis based on peak area.

Sample preparation and calibration

A stock solution of drug having a concentration of 1 mg/ml (i.e.1000 µg/ml) was prepared by dissolving in 10 ml methanol. Aliquots of the stock solutions were further diluted in methanol to get 100 µg/ml followed by filteration with 0.45 µm nylon filter and scanned in the wavelength range 400–200 nm. Spectra are presented in Figure 2. The maximum wavelength (λ_{max}) of ondansetron hydrochloride (OH) was obtained at 254 nm. 2, 4, 6, 8, 10, and 12 µl of second stock solution were spotted in triplicate on TLC plate to obtain concentrations of 200, 400, 600, 800, 1000, and 1200 ng/spot of ondansetron hydrochloride respectively. A calibration curve was obtained by plotting peak area against the corresponding concentration and linear least-square regression analysis was performed.

Analytical method development

The method of analysis was validated as per the recommendations of ICH Q2 (R1)[17] and USP[18] for the parameters like accuracy, linearity, precision, detection limit, quantitation limit, recovery, and robustness.

Precision

Precision was performed in two stages, namely system repeatability and method repeatability. For system repeatability, sample application and measurement of peak areas were carried out by analyzing six replicates of two concentrations (400 and 800 ng/spot) on the same day. For method repeatability, intra- and inter-day variations were studied for three different concentration levels of ondansetron (600, 800, and 1000 ng/spot).

Limit of detection and quantification

In order to determine detection and quantification limits (LOD and LOQ), ondansetron drug concentration in the lower part of calibration curve was used. Ondansetron solutions of 10, 20, 40, and 60 µg/ml were prepared and applied in triplicate (10 µl each). The amount of drug concentration versus average response (peak area) was plotted and the equation for this curve was determined. The standard deviations (SD) of responses were calculated. The average of standard deviations was calculated (ASD). Detection limit was calculated by (3.3×ASD)/*b* and quantification limit was calculated by (10×ASD)/*b*, where *b* corresponds to the slope obtained in the linearity study of method.

Recovery studies

The sublingual tablets formulation was first analyzed by the proposed method. The analyzed samples were spiked with 50%, 100%, and 150% of the standard drug and the mixture was re-analyzed. The experiment for each recovery sample was carried out six times to check the recovery of the drug at different levels in the formulations.

Robustness

Robustness of the developed method was determined by introducing small changes in the mobile phase composition and the effect on the result was examined. Mobile phases consisting of different compositions of chloroform and ethyl acetate with the same methanol and ammonia concentration (8.8 : 5.2 : 3.5 : 0.1 *v*/*v* and 9.2 : 4.8 : 3.5 : 0.1 *v*/*v*) were used. Robustness of the method was evaluated at two different concentration levels of 200 and 400 ng/spot.

Analysis of the ondansetron hydrochloride in sublingual tablets formulation

Ondansetron hydrochloride sublingual tablets were prepared by direct compression method using granulated mannitol as carrier material, cross-linked polyvinylpyrrolidone as disintegrant, silicified microcrystalline cellulose as the binder and magnesium stearate as lubricant. To determine the content of ondansetron in sublingual tablet prepared in house (Label Claim: 5 mg/tablet), ten tablets were weighed and powdered. Powder equivalent to 5 mg of ondansetron was weighed accurately and extracted with 10 ml of methanol. The solution was sonicated for 30 min and resulting solution was centrifuged at 3000 rpm for 5 min and filtered using syringe filter 0.45µ pore size. Final concentration of 500 µg/ml was obtained and 1 µl of this solution was spotted on the TLC plate, followed by development and scanning as described in *Materials and methods* section. The analysis was repeated in triplicate and possibility of excipients interference was observed.

Results and discussion

Optimization of mobile phase

The TLC procedure was optimized with a view to develop a method to quantify ondansetron in sublingual formulations. Chloroform was selected as one of the components of the mobile phase with acceptable resolution. However, the R_f value was too low, so the solvent strength was increased by adding polar solvents. Ethyl acetate and methanol was added to chloroform and the chromatograms were developed. The mobile phase comprising of chloroform : ethyl acetate : methanol (9 : 5 : 4 *v*/*v*) showed good resolution with $R_f = 0.52$ for ondansetron but tailing was observed and the spot of ondansetron was slightly diffused. Addition of ammonia improved the characteristics of the spot and its optimum quantity was 0.1 ml. The final mobile phase selected was a mixture of chloroform : ethyl acetate : methanol : ammonia (9 : 5 : 4 : 0.1 *v*/*v*), which gave a well-defined symmetrical peak of ondansetron at $R_f = 0.52 \pm 0.02$ (Figure 1a) which was visible under short wavelength (254 nm) ultraviolet light (Figure 2).

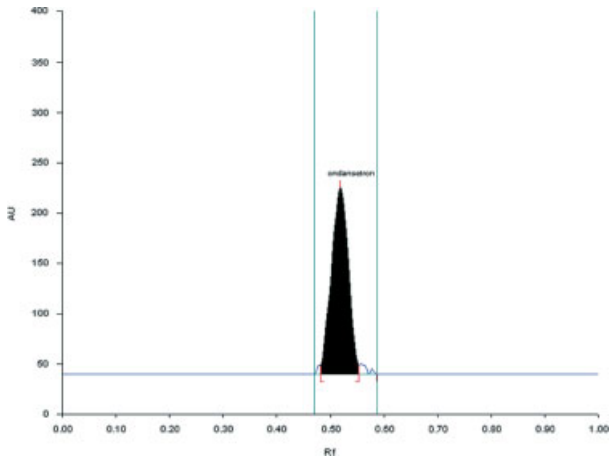

Figure 1(a) A typical HPTLC chromatogram of standard ondansetron (400 ng/spot, Rf = 0.52 ± 0.02). The mobile phase consisted of chloroform : ethyl acetate : methanol : ammonia (9 : 5 : 4 : 0.1 v/v).

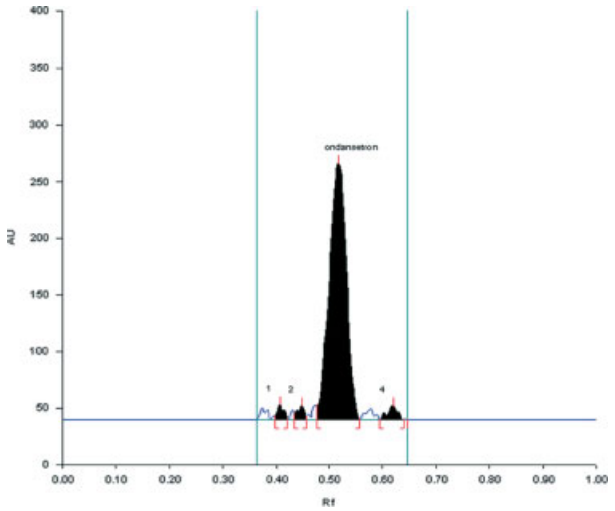

Figure 1(b) Chromatogram of ondansetron and the excipients in sublingual tablets formulation.

Calibration curves

The linear regression data for the calibration curves (n = 3) shown in Table 1 indicated a good linear relationship in the concentration range of 200–1200 ng/spot with respect to the peak area. No significant difference was observed in the slopes of the standard curves (ANOVA, $p < 0.05$).

Method validation

Precision

The repeatability of sample application and peak areas measured were expressed in terms of %RSD and the results revealed good system repeatability, intra- and inter-day precision (Table 2). The %RSD for system repeatability of sample application (400 and 800 ng/spot) was measured and found to be 0.99 and 0.43, respectively. This indicated that the system performance was very good and suitable for ondansetron analysis. The measurement of peak area at three different concentration levels (800, 1000, and 1200 ng/spot) showed low values of %RSD for inter- and intra-day variations, suggesting that the method had excellent precision.

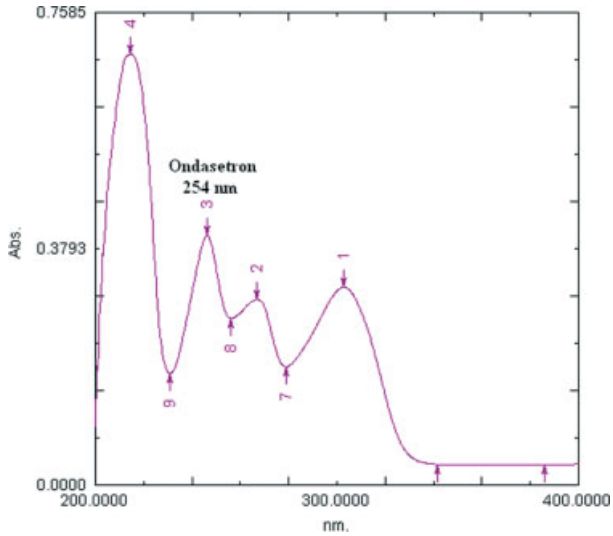

Figure 2. UV spectra of ondansetron Hydrochloride.

Table 1. Linear regression data for the calibration curves (n = 3)

Parameters	With Respect to the Mean Area
Linearity range (ng per spot)	200–1200
Correlation coefficient ± SD	0.9952 ± 0.0011
Slope ± SD	28.641 ± 0.32
Confidence limit of slope[a]	28.278–29.003
Intercept[b] ± SD	399.11 ± 5.21
Confidence limit of intercept[b]	393.21–405.01

[a] 95% confidence limit.
[b] Percentage of bias of intercept = −0.014.

Limit of detection and quantification

LOD and LOQ were calculated by the method described previously. The calibration curve in this study was plotted between amount of analyte versus average response (peak area) and the regression equation was obtained ($Y = 3.84X + 123.44$) with a regression coefficient of 0.9906. Therefore LOD and LOQ were found 14.83 and 44.92 ng, respectively, which indicated adequate sensitivity of the method.

Recovery studies

The accuracy of the method was evaluated by percentage recovery (by standard addition) of the drug. The proposed method was used for extraction and subsequent estimation of ondansetron from the sublingual tablets formulation after spiking known amounts of the drug (50, 100, and 150%) into the formulation. Accuracy was determined based on the amount of drug recovered. The results are shown in Table 2 and it was concluded that the present method was accurate for the estimation of ondansetron in pharmaceutical dosage forms.

Robustness

The results of robustness was carried out at two different concentrations levels of 200 ng and 400 ng/spot of ondansetron HCl in triplicate with %RSD value of 0.82 and 0.95. The low values of

Table 2. Validation parameters. System repeatability (n = 6)

Amount (ng/spot)	SD	%RSD[a]
System repeatability (n = 6)		
400	18.03	0.99
800	12.02	0.43
Intra-day precision (n = 6)		
800	41.11	1.43
1000	51.38	1.47
1200	39.86	0.97
Inter-day precision (n = 6)		
800	22.94	0.77
1000	29.46	0.79
1200	28.85	0.69
Recovery (n = 6)		

Excess drug added to the analyte (%)	Theoretical content (ng)	Recovery (%)	%RSD[a]
0	400	100.21	0.84
50	600	99.54	1.04
100	800	98.93	0.68
150	1000	101.16	0.72
Robustness of the method (n=3)			

	Mobile Phase Composition (Chloroform: ethyl acetate: methanol: ammonia)	
Concentration (ng/spot)	(8.8: 5.2: 3.5: 0.1) %RSD[a]	(9.2: 4.8: 3.5: 0.1) %RSD[a]
200	1.236	0.823
400	1.265	0.948

[a] Relative standard deviation,

%RSD obtained after introducing small changes in mobile phase composition indicated robustness of the method as indicated in Table 2. There was no significant variation in the slope values (ANOVA, $P < 0.05$).

Analysis of the prepared formulation

When the formulation was analysed, four spots at R_f 0.33, 0.45, 0.52 and 0.63 (Figure 1b) were observed in the chromatogram. The other spots might belong to the excipients present in the formulation. The drug content was found to be 99.19% with a %RSD of 0.462, respectively. The low %RSD value supported the suitability of this method for routine analysis of ondansetron in the novel drug delivery system.

Conclusion

The introduction of HPTLC estimation in pharmaceutical analysis represents a major step in terms of quality assurance. The developed HPTLC technique is precise, specific, and accurate. Statistical analysis indicated that the method is repeatable and selective for the analysis of ondansetron in pharmaceutical formulations with no interference from excipients. The method can be used to determine the purity of drug available from various sources by analyzing it for related substances and impurities.

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References

[1] M. Marty, P. Pouillart, S. Scholl, J. P. Droz, M. Azab, N. Brion, *et al*. Comparison of 5HT 3 (serotonin) antagonist ondansetron (GR38032F) with high dose metoclopramide in the control of cisplatin-induced emesis. *N. Engl. J. Med.* **1990**, *322*, 816.

[2] P. De Mulder, C. Seynaeve, J. Vermorken, P. A. van Liessum, S. Mols-Jevdevic, E.L. Allman, *et al*. Onansetron compared with high-dose metoclopramide in the prophylaxis of acute and delayed cis-platin-induced nausea and vomiting–a multicentre randomised, double-blind, crossover study. *Ann. Intern. Med.* **1990**, *113*, 834.

[3] T.J. Priestman. Clinical studies with ondansetron in the control of radiation-induced emesis. *Eur. J. Cancer Clin. Oncol.* **1989**, *25*(1), 29.

[4] W. D. Figg, G. E. Dukes, J. F. Pritchard, D. J. Hermann, H. R. Lesesne, S. W. Carson, *et al*. Pharmacokinetic of ondansetron in patients with hepatic insufficiency. *J. Clin. Pharmacol.* **1996**, *36*, 206.

[5] *The United State Pharmacopoeia*, 24th ed. National Publishing: Philadelphia, **2000**, pp. 1218.

[6] Government of India, Ministry of Health and Family Welfare, in *Indian Pharmacopoeia, Vol. II*. The Controller of Publications: New Delhi, **1996**, 2062.

[7] M. Deport, S. Leroux, G. Caille. High resolution liquid chromatographic method using ultraviolet detection for the determination of ondansetron in human plasma. *J. Chromatogr. B* **1997**, *693*, 399.

[8] P. Colthup, C.C. Felgat, J. Palmar, N.L. Scully. Determination of ondansetron in plasma and its pharmacokinetic in young and elderly. *J. Pharm. Sci.* **1991**, *80*, 868.

[9] S.P. Sastry, T. Rao. Spectrophotometric determination of amiodarone and ondansetron in pharmaceutical dosage forms with citric acid-acetic acid anhydride reagent. *Indian J. Pharm. Sci.* **2002**, *64*, 482.

[10] P.B. Raval, M. Puranik, S.J. Wadher, P.G. Yeole. A validated HPTLC method for determination of ondansetron in combination with omeprazole or rabeprazole in solid dosage form. *Indian J. Pharm. Sci.* **2008**, *70*, 386.

[11] B.H. Darji, N.J. Shah, A.T. Patel, N.M. Patel. Development and validation of a HPTLC method for the estimation of cefpodoxime proxetil. *Indian J. Pharm. Sci.* **2007**, *69*, 331.

[12] J. Novakovic, K. Nesmerak, H. Nova, K. Filka. An HPTLC method for the determination and the purity control of ciprofloxacin HCl in coated tablets. *J. Pharmaceut. Biomed. Anal.* **2001**, *25*, 957.

[13] E.E. Muller, J. Sherma. Quantitative hptlc determination of diphenhydramine hydrochloride in tablet, gelcap, and capsule antihistamine pharmaceuticals. *J. Liq. Chromatogr. Relat. Technol.* **1999**, *22*, 153.

[14] A.P. Argekar, J.G. Sawant. Simultaneous determination of atenolol and nitrendipine in pharmaceutical dosage forms by hptlc. *J. Liq. Chromatogr. Relat. Technol.* **1999**, *22*, 1599.

[15] D. DiGregorio, J. Sherma. Determination of the sleep aid ingredients diphenhydramine hydrochloride and doxylamine succinate in pharmaceutical products by quantitative hptlc. *J. Liq. Chromatogr. Relat. Technol.* **1999**, *22*, 1599.

[16] S. Chopra, F. J. Ahmad, R. K. Khar, S. K. Motwani, S. Mahdi, Z. Iqbal, *et al*. Validated high-performance thin-layer chromatography-methodfor determination of trigonelline inherbal extract and pharmaceutical dosage form. *Anal. Chim. Acta* **2006**, *577*, 46.

[17] ICH-Q2 (R1), Validation of Analytical Procedures: text and methodology. International Conference on Harmonization, Geneva, **2005**.

[18] *United States Pharmacopoeia/National Formulary*, 24th ed. Pharmacopeial Convention: Rockville, MD, **2000**, pp. 2149.