INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

SIMULTANEOUS DETERMINATION OF ONDANSETRON HYDROCHLORIDE AND OMEPRAZOLE IN TABLETS BY PLANAR CHROMATOGRAPHY

Lobhe GA*., Banerjee SK., Shirkhedkar AA. and Surana SJ

VJSM's Vishal Institute of Pharmaceutical Education & Research, Ale, Pune (Dt.), Maharashtra, India.

*Corresponding Author: globhe@gmail.com

ABSTRACT

A new simple, precise and selective high performance thin layer chromatographic (HPTLC) method has been developed and validated for simultaneous determination of ondansetron hydrochloride and omeprazole in tablets. Identification and determination were performed on 20 cm × 10 cm aluminium-backed TLC plates, coated with 0.2 mm layers of silica gel 60 F_{254}, previously washed with methanol. Ethyl acetate – methanol – ammonia, (11:3.5:0.2, *v/v*) as mobile phase. Detection was carried out densitometrically using a UV detector at 310 nm. Calibration curve were plotted in the range 400 – 1200 ng/spot (r = 0.999) for ondansetron hydrochloride and 1000 – 3000 ng/spot (r = 0.999), for omeprazole. The suitability of this HPTLC method for quantitative determination of these compounds is proved by validation in accordance with the requirements of ICH Guidelines.

Keywords: Ondansetron hydrochloride, Omeprazole, HPTLC.

INTRODUCTION

Ondansetron hydrochloride, 1, 2, 3, 4 – tetrahydro-9-methyl-3-(2-methylimidazol-1-yl methyl) carbazol-4-one hydrochloride[1], is a selective 5HT3 receptor antagonist. It is acarbazole which causes antinausent and antiemetic effects by selective and competitive blockade of the 5 HT3 receptors[2, 3]. The usual dose of ondansetron hydrochloride is 20 or 40 mg[4]. Omeprazole, (RS)-5-methoxy-2-[4 – methoxy-3, 5 dimethyl pyridin-2-yl) methyl] sulphinyl]-1H-benzimidazole[5], is substituted benzimidazole sulfoxides that function as proton pump inhibitors[6]. It is antisecretory drug effective for rapid healing peptic ulcer and corrosive oesophagitis[7, 8]. Structures of the drugs are shown in **Figure 1**.

In literature survey, UV spectrophotometry[9] and some HPLC methods[10-14] are reported for determination of ondansetron hydrochloride in pharmaceutical formulations and biological fluids. There are various methods such as chromatographic[15-21] for determination of omeprazole in from bulk drug and pharmaceutical formulations in combination with other drugs. In present paper, we describe a reliable, rapid and accurate HPTLC method for determination of ondansetron hydrochloride and omeprazole in tablets. The proposed HPTLC assays were validated in accordance with ICH guidelines (Q2B)[22].

EXPERIMENTAL

Instrument

HPTLC was performed with a Camag (Muttenz, Switzerland) Linomat V applicator, a Camag twin-trough TLC chamber, a Camag TLC scanner 3, Camag Wincats software, and a Hamilton (Reno, Nevada, USA) syringe (100 µL).

Solvents and chemicals

Ondansetron hydrochloride and omeprazole were kindly supplied as a gift sample by Torrent pharmaceuticals Ltd., Ahemadabad. Ethyl acetate, methanol and ammonia were used as solvents to prepare the mobile phase.

All the reagents used were of analytical reagent grade (S.D. Fine. Chemicals, Mumbai, India) and used without further purification.

Standard stock solutions

A combined stock solution containing 1mg/ mL ondansetron hydrochloride and 1 mg/ mL omeprazole was prepared in methanol. Calibration solutions were prepared by diluting the stock solution, to enable application of 400 to 1200 ng for ondansetron hydrochloride and 1000 to 3000 ng for omeprazole.

Sample Preparation

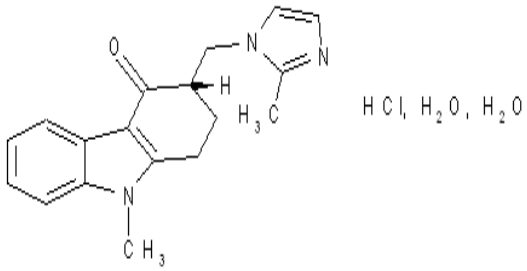
Twenty tablets were weighed and powdered in a glass mortar. An amount of powder equivalent to 40 mg ondansetron hydrochloride and 100 mg omeprazole was transferred to a 100 mL calibrated volumetric flask and extracted with methanol for 10 min by shaking mechanically. The solution was diluted to volume with the same solvent and filtered. This solution (2 µL, containing 800 ng ondansetron hydrochloride and 2000 ng omeprazole) was spotted for assay of ondansetron hydrochloride and omeprazole.

Mobile phase

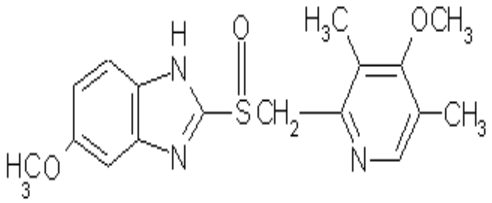
Ethyl acetate – methanol – ammonia, (11:3.5:0.2, v/v) was used as mobile phase.

Chromatographic condition

Chromatography was performed on 20 cm × 10 cm aluminium-backed TLC plates, coated with 0.2 mm layers of silica gel 60 F_{254} (E. Merck, Darmstadt, Germany), previously washed with methanol and stored in desicator. Samples were applied to the plates as 6 mm bands, 18.8 mm apart, 10 mm from the lower edge, by means of a Linomat V applicator (Camag, Muttenz Switzerland) equipped with a Hamilton syringe (Bonaduz., Switzerland). The rate of application was 15 s µL. ascending development of the plates to a distance of 70 mm was performed at 25 ± 2 ^{0}C with ethyl acetate – methanol – ammonia, (11:3.5:0.2, *v/v*), as mobile phase, in a Camag 20 cm × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland); previously saturated for 30 min with 14.7 mL mobile phase. The average development time was 20 min. After development, plates were dried. Densitometric scanning was performed on Camag TLC Scanner 3 in the reflectance-absorbance mode at 310 nm for all measurements and operated by Wincats software version 1.3.0 supplied by Anchrom technologists, (Mumbai). The source of radiation utilized was deuterium lamp, continues emits UV spectrum between 200 nm to 400 nm. The slit dimensions were 6.00 mm × 0.45 mm. Evaluation was via peak area with linear regression.



Ondansetron Hydrochloride



Omeprazole

Fig. 1: Structures of ondansetron hydrochloride and omeprazole

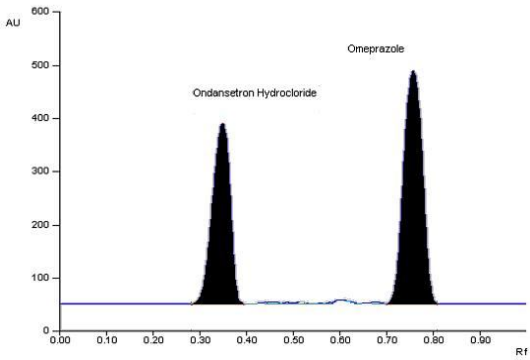


Fig. 2: Densitogram of standard ondansetron hydrochloride (400 ng/spot): peak 1 (R_F 0.35±0.02) and omeprazole (1000 ng/spot): peak 2 (R_F 0.76±0.02), in ratio of (1:2.5) measured at 310 nm, mobile phase ethyl acetate: methanol: ammonia (11:3.5:0.2, *v/v*). (Typical HPTLC Chromatogram of Ondansetron hydrochloride and Omeprazole)

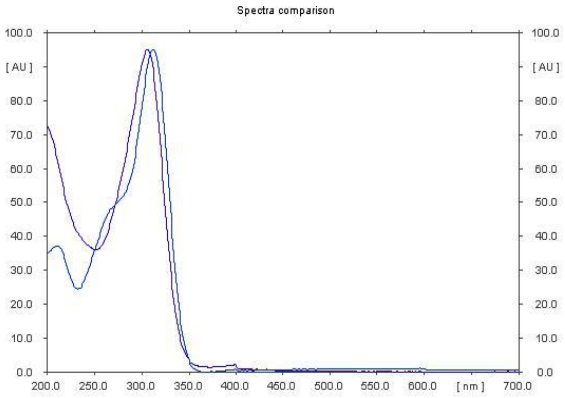


Fig. 3: Typical overlay spectra of standard 1 ondansetron and 2 omeprazole drug solutions

RESULTS AND DISCUSSION

Chromatography

The densitogram of standard ondansetron hydrochloride (400 ng/spot) and omeprazole (1000 ng/spot) was measured at 310 nm. The mobile phase ethyl acetate: methanol: ammonia (11:3.5:0.2, *v/v*) was selected because it gave high resolution, minimum tailing and R_f values of 0.35 and 0.76 for ondansetron hydrochloride and omeprazole, respectively **Figure 2**.

System suitability

According to the USP 28, method 621, system suitability tests are an integral part of a chromatographic analysis and should be used to verify that the resolution and reproducibility of the chromatographic system are adequate for the analysis. To ascertain effectiveness of the method developed in this study, system suitability tests were performed on freshly prepared standard stock solutions of ondansetron hydrochloride and omeprazole.

Linearity

Mix standard solutions containing 400, 600, 800, 1000 and 1200 ng/spot of ondansetron hydrochloride and 1000, 1500, 2000, 2500 and 3000 ng/spot of omeprazole were applied to the prewashed TLC plates. The plates were developed, dried and scanned as described above. The calibration graphs were constructed by plotting peak area against amount of drug (ng/spot). The results of optical and regression characteristics are shown in **Table 1.**

Sensitivity

In the proposed method, sensitivity of measurement for ondansetron hydrochloride and omeprazole was determined in terms of Limit of Quantitation (LOQ) and Limit of Detection (LOD). These were calculated by the use equation LOD = 3.3 x N / B and LOQ = 10 x N / B, where 'N' is standard deviation of the peak areas of the drugs (n = 3), taken as a measure of noise, and 'B' is the slope of the corresponding calibration plot. The LOQ and LOD for ondansetron hydrochloride was 102 ng and 33 ng, respectively [where N = 119.41, B = 11.631]. For omeprazole, the LOQ and LOD was found to be 226 ng and 74 ng, respectively [where N = 100.12, B = 4.423].

Precision

Precision was studied by use of standard solutions containing both drugs at concentrations covering the entire calibration range. Precision of the method, as intra-day variation (%CV) was determined, by analyzing ondansetron hydrochloride and omeprazole standard solutions, three times on the same day. Inter-day precision (%CV) was assessed by analyzing the same solutions on three different days over a period of one week. The results of the precision studies are as shown in **Table 2.**

Accuracy

The accuracy of the method was determined by multiple level recovery studies, i.e. use of standard additions at three different levels. Sample stock solution containing 800 ng mL^{-1} ondansetron hydrochloride and 2000 ng mL^{-1} omeprazole was prepared from a tablet formulation and spiked with amounts equivalent to 80, 100 and 120% of the amounts of drugs in the original solution. When these solutions were analysed the recoveries were found to be within acceptable limits **Table 3.**

Specificity

The mobile phase designed for the method resolved both drugs very efficiently, as shown in **Figure 2.** The R_F values of ondansetron hydrochloride and omeprazole were 0.35 and 0.76, respectively. Typical absorption spectra of ondansetron hydrochloride and omeprazole are shown in **Figure 3.** The peak purity was tested for both ondansetron hydrochloride and omeprazole by correlating spectra acquired at the start (S), apex (A), and end (E) positions of the peaks. Correlation between

these spectra were indicative of the purity of both the ondansetron hydrochloride peak (**Figure 4**; correlation r (S, M) = 0.999, r (M, E) = 0.999) and the omeprazole peak (**Figure 5**; correlation r (S, M) = 0.999, r (M, E) = 0.999). Therefore, it can be concluded that no impurities or degradation products coeluted with the peaks obtained from standard drug solutions.

Robustness

Robustness is a measure of the capacity of a method to remain unaffected by small but deliberate variations in the method conditions, and is an indication of the reliability of the method. Robustness was assessed by changing the migration distance of the solvent system. Typical results from ruggedness and robustness studies are as shown in **Table 4 & 5.**

Repeatability

Repeatability of sample application was assessed by spotting 10 μL of drug solution 7 times on a TLC plate then developing the plate and recording peak height and peak area for the spots. The chromatographed spot was scanned seven times without changing height and area for ondansetron hydrochloride and omeprazole, respectively.

Stability studies

To test the stability of drugs on the TLC plates, analytes were tested against freshly prepared solutions. No decomposition of the drug was observed during chromatogram development. No decrease in the concentration of drugs on the plate was observed within three hours. A decrease in the amount of ondansetron hydrochloride and omeprazole on the plate was observed after twenty four hours. Chromatograms should therefore be scanned within three hours of development. The standard drug solutions were found to be stable at room temperature in the solvent (methanol) used to prepare the solutions. This stability of the analyte in the solvent was assessed by investigating three samples of each drug solution at high and low concentrations. The results of the stability studies are listed in **Table 6.**

CONCLUSION

This method was developed for the first time on HPTLC to estimate the two drugs in formulation, in order to analyse more samples at a time. The method is easy to perform, precise and accurate. The whole procedure may be extended to pharmaceutical preparation.

ACKNOWLEDGEMENTS

The authors are grateful to R.C. Patel College of Pharmacy, Shirpur, India for providing the instrumental facilities.

Table 1: Results of optical and regression characteristics

Parameters	Ondansetron hydrochloride	Omeprazole
Concentration Range	400-1200	1000-3000
LOD (ng/spot)	33	74
LOQ (ng/spot)	102	226
Regression Equation	11.631x +3667.5	4.423x +7292.2
Correlation Coefficient	0.9992	0.9994

Table 2: Results from determination of the precision of analysis of ondansetron hydrochloride and omeprazole

Drug	Conc. [ng/spot]	Intra-day precision		Inter-day precision	
		Mean ± S.D.	% *RSD* [*n* = 3]	Mean ± S.D.	% *RSD* [*n* = 3]
Ondansetron hydrochloride	600	598.60 ± 1.38	0.23	600.90 ± 5.73	0.95
	800	796.23 ± 3.07	0.38	796.52 ± 2.94	0.36
	1000	998.91 ± 3.71	0.37	1001.21 ± 5.53	0.55
Omeprazole	1500	1493.65 ± 2.58	0.17	1487.10 ± 4.69	0.31
	2000	1992.26 ± 7.21	0.36	2001.1 ± 10.46	0.52
	2500	2494.41 ± 9.43	0.37	2482.7 ± 12.61	0.50

Table 3: Results of recovery studies

Drug	Amount Recover [ng]	Amount recovered ± S.D. [ng] (n = 3)	% Recovered	%RSD
Ondansetron hydrochloride	0	792.01 ± 1.21	99.00	0.15
	80	640.11 ± 1.57	100.01	0.24
	100	798.52 ± 3.01	99.81	0.37
	120	959.41 ± 0.32	99.93	0.32
Omeprazole	0	1992..78 ± 6.85	99.63	0.34
	80	1596.08 ± 5.65	99.75	0.35
	100	1999.04 ± 2.68	99.95	0.13
	120	2398.44 ± 4.47	99.93	0.18

Table 4: Results of ruggedness studies

	Amount of Ondansetron hydrochloride Found [%]	%RSD (n=5)	Amount of Omeprazole Found [%]	%RSD (n=5)
Analyst I	99.63	0.46	100.29	0.30
Analyst II	99.56	0.52	100.05	0.21

Table 5: Results from robustness studies

Development distance [cm]	Ondansetron hydrochloride (4 mg) [%]	Omeprazole (10 mg) [%]
7.0	99.13	99.43
7.5	99.82	99.89
8.0	100.06	99.54

Table 6: Results of stability studies

Drug	% Drug loss [%RSD]		
	3 h	24 h	48 h
Ondansetron hydrochloride	No loss	1.37 ± 0.32	2.65 ± 0.20
Omeprazole	No loss	1.26 ± 0.37	2.96 ± 0.79

REFERENCES

1. Moffat AC and Widdop B. Clarke's Analysis of Drugs and Poisons, 3rd edn, Vol. 1, Pharma Press. 2004:1368-1369.
2. The Merck Index - An Encyclopedia of Chemicals, Drugs and Biological,13th edn, Merck and Company, Inc USA. 2001:1225.
3. The United States Pharmacopoeia, United States Pharmacopoeia Convention, Inc., Rockville, 27th revision. 2005:1418-1421.
4. British Pharmacopoeia, Vol. I, HMSO, Cambridge, International edn. 2004:1460-1462 .
5. European Pharmacopoeia, The Council of Europe, 3rd edn, ISBN, France. 1997:2149-2149.
6. The Indian Pharmacopoeia, Government of India, Ministry of Health and Family Welfare, Delhi Vol. I. 1996:532-534.
7. Martindale-The Complete Drug Reference, 33rd edn, Pharmaceutical Press, London, Chicago. 2002:1241.3.
8. Goodman and Gilman's, The Pharmacological Basis of Therapeutics, 10th edn, Mc Graw Hill, Medical Publishing Division. 2001:1007.

9. Siluveru M and Stewart JT. J Chromatogr B. 1997;691:217-222.
10. Depot M, Leroux S and Caille G. J Chromatogr B. 1997;693:399-406.
11. Dotsikas Y, Kousoulos C, Tsatsou G and Loukas YL. J Chromatogr B. 2006;836:79-82.
12. Liu JT and Stewart. J Chromatogr B. 1997;694:179-184.
13. Rao TS, Rao PSNHR, Prasad UV and Sastry CSP. Asian J Chemistry. 2002;14(1):217-222.
14. Krishnaiah YSR, Lakshmi M, Satyanarayan V and Bhaskar P. Asian J Chemistry. 2000;14(3-4):1246-1250.
15. Perez-Ruiz T, Martinez Lozano C, Sanz A and Brovo E. Raquel Galera. J Pharm Biomed Anal. 2006;42:100-106.
16. Rezk NL, Brown KC and Angela DM. J Chromatogr B. 2006.
17. Jia H, Li Wei and Zhao K. J Chromatogr B. 2006;837:112-115.
18. Hitochi MM, Satoh TS, Habuchi T and Suzuki T. J Pharm Biomed Anal. 2006;41:565-570.
19. Della Greca M, Iesce MR, Previtera L, Rubino M, Temusi F and Brigante M. Chemosphere. 2006;63:1087-1093.
20. Shimizu MT, Uno, Niioko T, Yaui-Furukoni N, Takanata Kazunobu T and Tomonori Tateshi S. J Chromatogr B. 2006;832:241-248.
21. Hofmann U, Gerd MS and Klotz TU. J Chromatogr B. 2006;831:85-90.
22. ICH –Guidelines Q2B, Validation of Analytical Procedures: Methodology (CPMP/ICH/281/95) November (1996) Geneva, Switzerland.