A High-Performance Thin Layer Chromatography (HPTLC) Method for Simultaneous Determination of Diphenhydramine Hydrochloride and Naproxen Sodium in Tablets

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ABSTRACT: A rapid and simple high-performance thin layer chromatography (HPTLC) method with densitometry at 230 nm was developed and validated for simultaneous determination of diphenhydramine hydrochloride (DPH) and naproxen sodium (NPS) from pharmaceutical preparation. The separation was carried out on aluminum plates precoated with silica gel 60 F_{254} using mobile phase toluene:methanol:glacial acetic acid (7.5:1:0.2, v/v/v). The linearity range lies between 200 and 1200 ng/band for DPH and 1760 and 10,560 ng/band for NPS with correlation coefficients of 0.994 and 0.995, respectively. The R_f value for DPH is 0.20 ± 0.05 and for NPS is 0.61 ± 0.06. % Recoveries of DPH and NPS was in the range of 99.70%–99.95% and 99.63%–99.95%, respectively. Limit of detection value for DPH was 13.21 ng/band and for NPS was 8.03 ng/band. Limit of quantitation value for DPH was 40.06 ng/band and for NPS was 24.34 ng/band. The developed method was validated as per ICH guidelines. In stability testing, DPH was found unstable to acid and alkaline hydrolysis, and DPH and NPS were found unstable to oxidation, whereas both the drugs were stable to neutral and photodegradation. The proposed method was successfully applied for the routine quantitative analysis of dosage form containing DPH and NPS.

KEYWORDS: diphenhydramine hydrochloride, naproxen sodium, HPTLC, validation

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Introduction

Diphenhydramine hydrochloride (DPH) is chemically 2-benzhydryloxyethyldimethylamine hydrochloride (Fig. 1).[1] It is an antihistamine with anticholinergic (drying) and sedative effects. Antihistamines appear to compete with histamine for cell receptor sites on effector cell. It is very soluble in water and freely soluble in alcohol.[2]

Naproxen sodium. Naproxen sodium (NPS) is 2-(6-methoxynaphthalen-2-yl)propanoate sodium (Fig. 2).

It is a nonsteroidal anti-inflammatory drug commonly used for fever, inflammation, the reduction of moderate-to-severe pain, and stiffness. The main mechanism of NPS action, inhibition of prostaglandin synthesis, makes the drug effective in combating pain and inflammation. NPS is soluble in methanol and water (pKa 4.15).[3,4]

A combined dose tablet formulation containing DPH and NPS is available in the market for the treatment of pain and insomnia. The combination is not yet official in any of the pharmacopeia. Hence, there is no official method available for assay of combined dose formulation containing DPH and NPS. Literature survey revealed a few spectrophotometric,[5] high-performance liquid chromatography (HPLC),[6–8] and HPTLC[9,10] methods reported for the determination of DPH and NPS in combination with other drugs. There is only one HPLC method reported in literature for simultaneous determination of DPH and NPS in tablets. However, there is lack of such equipment in many resource limited countries. In poor countries, where such equipment is available, the high cost of HPLC grade solvents and columns and consumption of solvent significantly affect timely release of laboratory results for action.[11–18] Therefore, alternative methods are needed to facilitate and increase the speed of analysis, with relatively few costs. HPTLC has gained importance in pharmaceutical analysis because of its advantages such as advanced separation efficiency and detection limits, less cost per analysis and low

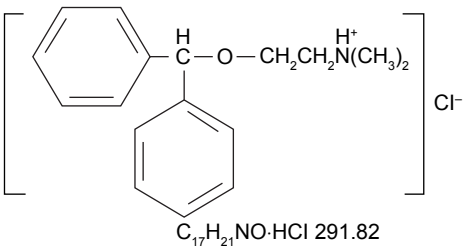

Figure 1. Chemical structure of DPH.

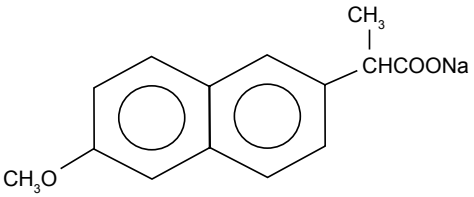

Figure 2. Chemical structure of NPS.

analysis time, not prior treatment for solvents like filtration and degassing, low mobile phase consumption per sample, no interference from previous analysis—fresh stationery phase and mobile phase for each analysis, and allowing parallel treatment of multiple samples during chromatography. This work was undertaken with an objective to develop a rapid, sensitive, economical, and less time-consuming HPTLC method, as an alternative to the reported methods, for routine quality control of pharmaceutical formulation containing DPH and NPS.

Materials and Methods

Chemicals and reagents. DPH and NPS in Pure form was received as a gift sample from Souvin Pharmaceuticals (I) Pvt. Ltd. and Rubicon Pharmaceuticals, respectively, and DPH and NPS in combination (claimed labeled amount 25 mg DPH and 220 mg NPS per tablet) in formulation. The solvents used in the chromatography such as toluene (Merck), methanol (Merck), and glacial acetic acid (Merck) were of AR grade.

Instrumentation. Microsyringe (Linomat syringe, Hamilton-Bonaduz Schweiz), precoated silica gel 60 F_{254} glass plates (10 × 10 cm with 200 µm thickness HPTLC; Merck), Camag Linomat V automatic sample applicator (Camag), Camag 100 µL sample syringe (Hamilton), Camag twin trough chamber 10 × 10 cm (Camag), UV chamber (Camag), TLC scanner III (Camag), and win CATS version 1.4.0 software (Camag) were used in this study.

Chromatographic conditions. Standard and sample solutions, 5 µL each, were applied on the TLC plate using Camag Linomat V automatic sample applicator in the form of band (bandwith: 6 mm, distance between two bands: 14 mm) using microsyringe. A constant application rate of 150 nL s^{-1} was used. The plates were saturated for 20 minutes in a twin trough glass chamber (for 10 × 10 cm) with the mobile phase of toluene:methanol:glacial acetic acid (7.5:1:0.2, v/v/v). The plates were then placed in the mobile phase, and ascending development was performed to a distance of 8 cm. Subsequent to the development, the plates were air dried and a densitometric scanning (slit dimensions: 5 × 0.45) was performed at 230 nm using Camag TLC scanner III operated in reflectance–absorbance mode.

Analysis of formulation.

Preparation of standard solution. Accurately weighed quantity of 10.0 mg of DPH and 88.0 mg of NPS was transferred to 25.0 mL volumetric flask; 20 mL methanol was added and ultrasonicated for 15 minutes; and volume was then made up to the mark with methanol. From the above solution, 3.0 mL of solution was diluted to 10.0 mL with methanol (concentration: 120 µg/mL of DPH and 1056 µg/mL of NPS).

Preparation of sample solution. Twenty tablets were weighed accurately; average weight was calculated, and the tablets were crushed to obtain fine powder. Accurately weighed quantity of tablet powder equivalent to about 10.0 mg of DPH and 88.0 mg of NPS was transferred to 25.0 mL volumetric flask; 20 mL methanol was added and ultrasonicated for 15 minutes; and volume was then made upto the mark with methanol. The solution was mixed and filtered through Whatman filter paper No. 42. From the filtrate, 3.0 mL of solution was diluted to 10.0 mL with methanol to give 120 µg/mL of DPH and 1056 µg/mL of NPS. A total of 5 µL of this solution is used for the determination.

Preparation of calibration curve. The working standard stock solution containing DPH and naproxen was prepared in methanol. A linear relationship between peak area and concentration was evaluated by making five measurements at six concentration levels over a range of 200–1200 ng/band for DPH and 1760–10,560 ng/band for NPS.

Method validation. The method was validated in compliance with ICH guidelines.

Accuracy. An accurately weighed quantity of a sample equivalent to ~10 mg DPH and 88 mg NPS was transferred individually in nine different 25.0 mL volumetric flasks, added 8/70.4 mg, 10/88 mg, and 12/105.6 mg of DPH/NPS to the sample for 80%, 100%, and 120% level of recovery, respectively. All dilutions were performed with methanol. Solutions were prepared in triplicate and analyzed. Accuracy was determined and expressed as % recovery.

Precision. To ascertain repeatability and reproducibility of the method, precision studies were performed. The sample solution was prepared and analyzed in the similar manner as described under analysis of formulation. Intraday precision was determined by analyzing a sample solution at three different time intervals on the same day, and interday precision was determined by analyzing a sample solution on three consecutive days.

Robustness. To evaluate the robustness of the proposed method, small but deliberate variations in the optimized method parameters were done. By introducing small changes in the mobile phase composition, mobile phase volume, duration of chamber saturation with mobile phase, time from spotting to development (five minutes, 20 minutes, and one hour), and time from development to scanning (five minutes, 20 minutes, and one hour), the effects on R_f value and peak area of drugs were examined. The composition of mobile phase was changed slightly (±0.1 mL for component). TLC plates with standard and sample bands were run with mobile phases of composition, toluene:methanol:glacial acetic acid (7.6:1.1:0.3, v/v/v and 7.4:0.9:0.1, v/v/v). Mobile phase volume and duration of chamber saturation were varied at 8.7 ± 1.0 mL (7.7, 8.7, and 9.7 mL) and 20 minutes ±25% (15, 20, and 25 minutes), respectively.

Limit of detection and limit of quantitation. The limit of detection (LOD) and limit of quantitation (LOQ) of the developed method were calculated using $3a/S$ and $10a/S$ phenomena for the limits of detection and quantification, respectively), where a is the standard deviation of the y-intercepts and S is the slope of the calibration curve.

Forced degradation studies. In forced degradation studies, intentional degradation was tried by exposing a sample to the following stress conditions: acidic (0.1 M HCl), alkaline (0.1 M NaOH), and oxidation (3% H_2O_2). For intentional degradation, contents of the flasks were refluxed in a water bath at 80°C for three hours. For heat and photo degradation, a sample was kept at 60°C and in UV light (254 nm) for 24 hours. After the respective time intervals, all the flasks were removed and allowed to cool. The samples were then prepared and analyzed in the similar manner as described under analysis of formulation.

Results and Discussion

This study was aimed at the development of sensitive, economical, and less time-consuming HPTLC technique for the determination of DPH and NPS in pharmaceutical dosage forms. Chromatography was performed on silica gel 60 F_{254} as stationery phase. The application of sample in the form of band, instead of spot, has certain advantages such as large quantities of sample can be handled for application, bands are easier to scan for, detector response is higher in the case of band, and maximizing quantitative accuracy. Hence, the samples were applied in the form of band. Chromatographic chamber saturation time was 20 minutes. Different scan settings are required to avoid distortion of recorded chromatogram. Hence, the slit dimension was adjusted to 5 × 0.45 as the size of sample band was 6 mm. The selection of wavelength was based on maximum absorbance for optimum sensitivity. Several trials were made using different solvents with varying polarity and in different proportions to obtain good resolution and sharp peaks with acceptable R_f values (0.2–0.8). Among the different mobile phase combinations tested, mobile phase consisting toluene:methanol:glacial acetic acid (7.5:1:0.2, v/v/v) gave better resolution and sharp peaks with R_f values of 0.20 ± 0.05 and 0.61 ± 0.06 for DPH and NPS, respectively. Figure 3 shows the HPTLC densitogram for mixed standard, containing DPH and NPS, using the optimum chromatographic conditions.

Table 1. Calibration parameters.

PARAMETERS	DPH	NPS
Linearity range (ng/band)	200–1200	1760–10560
Linearity equation	y = 1.496x + 27.46	y = 2.296x + 73.56
Correlation coefficient	0.994	0.995

Validation of the method. The developed method was validated in accordance with the ICH guidelines.

Linearity and range. Linearity was found in the range of 200–1200 ng/band for DPH and 1760–10,560 ng/band for NPS as shown in Table 1. The drug peak area was calculated for each concentration level, and a graph was plotted for drug concentration against the peak area. The calibration curves for DPH and NPS are depicted in Figures 4 and 5, respectively.

Accuracy (recovery study). The accuracy of the method was established using standard addition method. The known amount of standard was added at three different levels to preanalyzed tablet powder. Determination was performed in triplicate at each level. The results of recovery studies are expressed in terms of % recovery and are shown in Table 2. The % recovery for both DPH and NPS was found to be nearly 100% indicating that there is no interference in the analysis by the excipients present in the tablet formulation.

Precision. Repeatability and intermediate precision of the developed method were expressed in terms of percent relative standard deviation (RSD) of the peak area. Combined dosage form was analyzed at three levels of concentration of the assay at different time intervals on the same day. The interday precision study was performed by analyzing dosage form on three consecutive days. The RSD for repeatability (intraday precision) was found to be 0.4552% for DPH and 0.2934% for NPS. The RSD for intermediate (interday) precision was found to be 0.2903% for DPH and 0.1811% for NPS. The % RSD for intraday and interday precision is <2, indicating the precision of the method.

Robustness. The effect of change in mobile phase composition (±0.1 mL), in chamber saturation period (±25%), in time

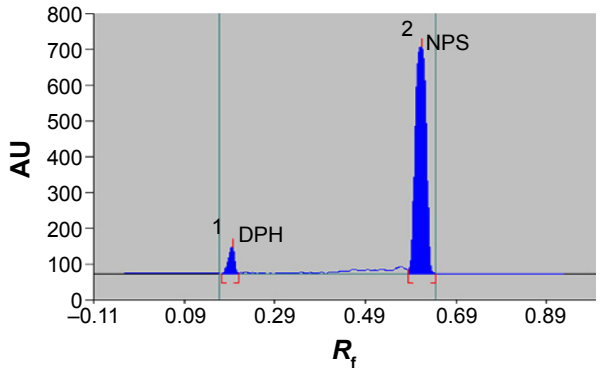

Figure 3. Typical densitogram of DPH and NPS.

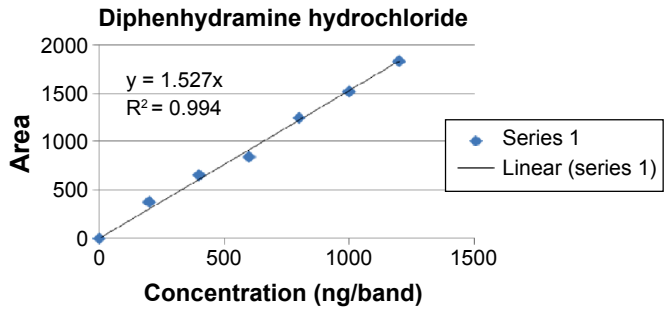

Figure 4. Standard calibration curve for DPH.

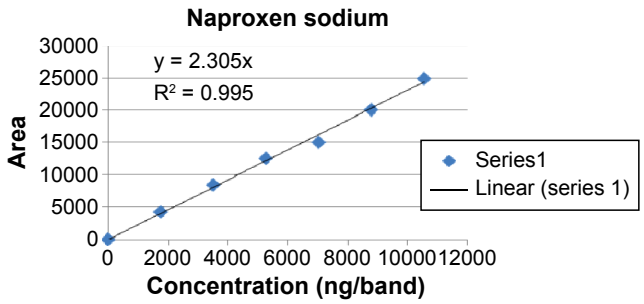

Figure 5. Standard calibration curve for NPS.

from application to development (five minutes, 20 minutes, and one hour), in time from development to scanning (five minutes, 20 minutes, and one hour), on the peak area, and in R_f value of drugs was studied. The method was found to be unaffected by small changes in method parameters with % RSD < 2 for peak area, and there was no significant change (less than ±0.05 R_f units) in R_f values for both the drugs. Hence, the developed method is considered to be robust.

LOD and LOQ. The LOD was found to be 13.21 ng/band for DPH and 8.03 ng/band for NPS. LOQ was found to be 40.06 ng/band for DPH and 24.34 ng/band for NPS. The LOD and LOQ values are in ng/band indicating the sensitivity of the method.

Analysis of formulation. Analysis of the formulation containing DPH (25 mg) and NPS (220 mg) was performed, and the results are expressed as % amount of the label claim. The content of DPH and NPS was found to be close to 100%, indicating that there is no interference in the analysis by the excipients likely to be present in the tablet matrix. The results are summarized in Table 3.

Forced degradation studies. DPH was found to degrade in acid, alkaline, and oxidation stress conditions employed. Maximum degradation was observed under alkaline stress condition. However, it was found stable in neutral, heat, and photodegradation stress conditions. NPS was found to degrade under oxidation stress condition, and it was stable under rest of the stress conditions employed. The % assay of active substance and the R_f values of degradation products are given in Table 4. Densitogram of acid, alkaline, and oxide treated samples are shown in Figures 6, 7, and 8, respectively. Densitogram of tablet treated under acidic condition (0.1 M HCl) showing peaks for unknown degradation product (1), DPH (2), and NPS (3).

Table 2. Accuracy results.

LEVEL OF RECOVERY	RECOVERY (%)*		S.D.		% R.S.D.	
	DPH	NPS	DPH	NPS	DPH	NPS
80%	99.95	99.95	±0.3819	±0.1174	0.38	0.11
100%	99.70	99.63	±0.6082	±0.0902	0.610	0.091
120%	99.77	99.90	±0.4119	±0.0360	0.41	0.04

Note: *Mean of three determinations.

Table 3. Assay result of formulation by HPTLC method.

FORMULATION	AMOUNT OF DRUG ESTIMATED* (mg/Tablet)		% LABEL CLAIM*	
	DPH	NPS	DPH	NPS
In-house tablet formulation (DPH = 25 mg & NPS = 220 mg)	24.91	218.43	99.65	99.28

Note: *Mean of six determinations.

Densitogram of tablet treated under alkali conditions (0.1 M NaOH) showing peaks for unknown degradation products (1 and 3), DPH (2), and NPS (4); moreover, densitogram of tablet treated under oxide condition (3% H_2O_2) showing peaks for unknown degradation product (1 and 3), DPH (2), and NPS (4). The developed method was able to selectively quantitate analyte peak in the presence of degradation products, indicating that the method can be employed as a stability indicating one.

Conclusion

The proposed HPTLC method gives well-resolved peaks for DPH and NPS. Based on the results obtained, it is concluded that the method is sensitive, accurate, precise and reproducible, economical, and less time consuming. The proposed method was able to selectively quantitate DPH and NPS in the presence of the degradation products, and hence can be considered as a stability indicating one. Hence, the proposed method can be used, as an alternative to the reported methods, for routine quality control of pharmaceutical formulations containing these drugs, alone or in combination.

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Table 4. Results of forced degradation study.

STRESS CONDITION	TEMPERATURE AND TIME	PERCENT ASSAY OF ACTIVE SUBSTANCE		R_f VALUE OF DEGRADED PRODUCT
		DPH	NPS	
Acid (0.1 M HCl)	80°C for 3 hr	74.87	99.38	0.10
Alkali (0.1 M NaOH)	80°C for 3 hr	72.12	100.50	0.10, 0.24
Oxide (3.0% H_2O_2)	80°C for 3 hr	80.62	65.20	0.09, 0.67
Neutral	80°C for 3 hr	99.28	99.58	–
Heat (60°C)	60°C for 24 hr	99.09	99.03	–
UV-exposure	254 nm for 24 hr	99.17	99.52	–

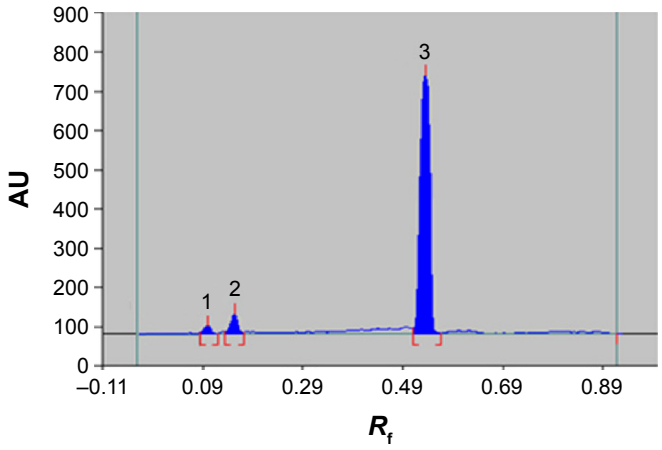

Figure 6. Densitogram of acid (0.1 M HCl) treated tablet sample.

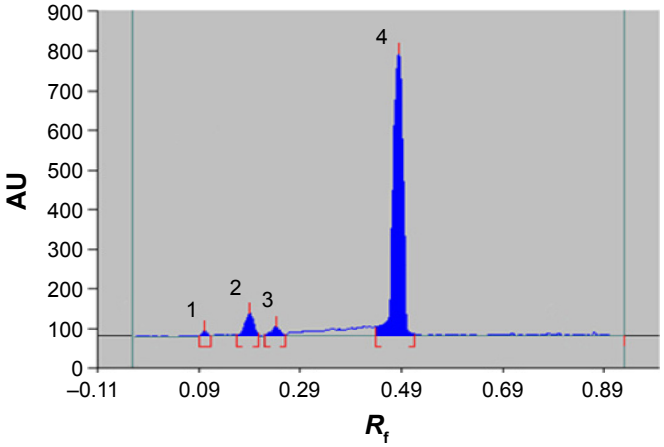

Figure 7. Densitogram of alkali (0.1 M NaOH) treated tablet sample.

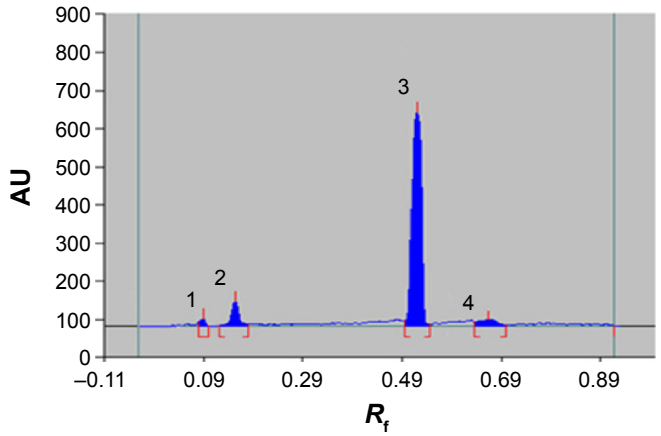

Figure 8. Densitogram of oxide (3% H_2O_2) treated tablet sample.

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Author Contributions

Conceived and designed the experiments: RPB and SSS. Analyzed the data: RPB and SBW. Wrote the first draft of the manuscript: SSS and RPB. Contributed to the writing of the manuscript: SBW and SSC. Agree with manuscript results and conclusions: RPB and SBW. Jointly developed the structure and arguments for the paper: RPB and SBW. Made critical revisions and approved final version: RPB and SSS. All authors reviewed and approved of the final manuscript.

REFERENCES

1. *Indian Pharmacopoeia*. Vol. 2. Government of India, Ministry of Health and Family Welfare, The Indian Pharmacopoeia Commission: Ghaziabad; 2007: 427.
2. Benadryl Allergy & Cold (Diphenhydramine Hydrochloride) Caplets Patient Information. McNeil-PPC, Inc. From McNeil-PPC website. Accessed February 21, 2008.
3. Kim YB, Park IY, Lah WR. The crystal structure of naproxen sodium (C14H13O3Na), a non-steroidal antiinflammatory agent. *Arch Pharm Res*. 1990; 13:166–173.
4. Segre EJ. Naproxen sodium (Anaprox): pharmacology, pharmacokinetics and drug interactions. *J Reprod Med*. 1980;25(4 suppl):222–225.
5. Trinath M, Banerjee SK, Hari Hara Teja D, Bonde CG. Development and validation of spectrophotometric method for simultaneous estimation of sumatriptan and naproxen sodium in tablet dosage form. *Pharm Sin*. 2010;1(1):36–41.
6. Ali NW, Abdelkawy M, Abdelhamid NS. Simultaneous determination of paracetamol and diphenhydramine hydrochloride mixture in the presence of their degradation products. *IOSR J Pharm Biol Sci*. 2013;6(5):44–52.
7. Ge ZK, Luo YH, Zhao XY, Zhang YJ, Zhang H, Tian CC. Simultaneous determination of ibuprofen and diphenhydramine HCl in orally disintegrating tablets and its dissolution by reversed-phase high performance liquid chromatography (RP-HPLC). *Afr J Pharm Pharmacol*. 2011;5(18):2100–2105.
8. Solanki SD, Patel PU. Development and validation of reversed-phase high performance liquid chromatographic method for simultaneous estimation of sumatriptan succinate and naproxen sodium in pharmaceutical dosage form. *Int J Pharm Pharm Sci*. 2012;4(1):276–278.
9. Parekh SP, Dedania ZR, Dedania R, Vijyendraswamy SM. Analytical method development and validation of HPTLC method for simultaneous estimation of sumatriptan succinate and naproxen sodium in pharmaceutical dosage form. *Int J Ayurveda Pharm Res*. 2014;2(3):94–99.
10. Pawar SM, Patil BS, Chaudhari RY. Validated HPTLC method for simultaneous quantitation of domperidone maleate and naproxen sodium in bulk drug and formulation. *Eurasian J Anal Chem*. 2010;5(3):284–292.
11. AOAC. *International Official Methods of Analysis*. 17th ed. Gaithersburg: 2003.
12. ICH (Q2A). Harmonized Tripartite Guideline, Text on Validation of Analytical Procedures. Geneva: IFPMA; 1994:1–5.
13. ICH (Q2B). Harmonized Tripartite Guideline, Validation of Analytical Procedures: Methodology. Geneva: IFPMA; 1996:1–8.
14. ICH (Q2R1). Harmonized Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology. Geneva: IFPMA; 2005:1–13.
15. Nash RA, Wachter AH. *Pharmaceutical Process Validation*. 3rd ed. New York: Marcel Dekker Inc; 2003.
16. Singh S, Bakshi M. Guidance on the conduct of stress tests to determine inherent stability of drugs. *Pharm Tech*. 2000;24:1–14.
17. Carstensen JT, Rhodes CT. *Drug Stability Principles and Practices*. 3rd ed. New York: Marcel Dekker Inc; 2005:370–374.
18. ICH (Q1AR2). Harmonized tripartite guideline, stability testing of new drug substances and products, IFPMA. In: Proceedings of the International Conference on Harmonization. Geneva; 2003.