



Simultaneous Estimation of Diphenhydramine Hydrochloride and Naproxen Sodium in Raw and Tablet Formulation by Reverse Phase - High-Performance Liquid Chromatographic Method

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Authors' contributions

This work was carried out in collaboration between both authors. Author MAB designed the study and wrote the protocol. Author GM performed the lab experiments and statistical analysis, managed the literature search and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: A simple, reproducible method was developed for the simultaneous determination of Diphenhydramine and Naproxen in tablet formulation using reverse phase HPLC method.
Methodology: HPLC separation was carried over on Thermo hypersil – keystone C18 (250 x 4.6 mm, 0.5 μ) isocratic mode column using a mobile phase comprising a 30:70 (% v/v) of methanol and 0.5 mM phosphate buffer. The detection was proceeded out by UV detector at 270 nm. The linearity range taken for Diphenhydramine and Naproxen were 15 – 35 mcg/ml and 100-500 mcg/ml respectively.
Results: The percentage recovery was found to be 99.74% and 101.11% for Diphenhydramine and Naproxen respectively. The amount of Diphenhydramine and Naproxen found in tablet was

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25.006 mg and 219.07 mg respectively. The proposed RP-HPLC method shows a good separation of Diphenhydramine and Naproxen, the retention time was found to be 3.1 minutes for Diphenhydramine and 4.9 minutes for Naproxen.

Conclusion: The proposed method shows very less time consuming analysis. The proposed method of analysis shows good separation at less retention time and less cost. The developed method can be successfully applied for the determination of Diphenhydramine and Naproxen in bulk powder and combined tablet dosage form.

Keywords: Diphenhydramine (DPH) and naproxen (NAP); RP-HPLC; UV; mobile phase.

1. INTRODUCTION

Diphenhydramine hydrochloride, DPH, (2-(diphenylmethoxy)-N,N-dimethylethylamine hydrochloride) (Fig. 1), belongs to the first generation anti-histamine drug [1]. DPH is the best drug of choice in many countries and DPH is very effective than some of current antihistamine drugs [2]. DPH is widely used as anti-allergic, antiemetic and has antitussive activity [3-5]. The DPH syrup and tablet formulation is used in cough suppression treatment [6,7]. The DPH is prescribed as an antiemetic drug with the combination of chemotherapy drug like Cisplatin [6]. The DPH has been used as sedative in dentistry and in local anesthesia for children's [2]. DPH is administered through oral route as tablet or syrup formulations [8,9]. Various studies show that DPH is not stable in syrup or elixir form but more stable in solid form [10]. Naproxen (NAP) chemically known as (+)-(S)-2-(6-methoxynaphthalen-2-yl) propanoic acid (Fig. 2). NAP is a (NSAID) non-steroidal anti-inflammatory drug, which has analgesic and antipyretic properties [11,12]. NAP is widely used for the treatment of reduction in pain, fever, inflammation osteoarthritis, kidney stones, rheumatoid arthritis, gout, menstrual cramps and tendinitis [12,13]. The Pharmacology of NAP is by decreasing the levels of prostaglandin in various fluids and tissues achieved by inhibiting the cyclooxygenase [2,12]. NAP poses a moderate risk of stomach ulcers compared to ibuprofen [14]. When compare to other NSAID drugs NAP have very fewer adverse effects [3,13]. NAP is formulated in tablets and suppositories [11].

Several methods like Spectrophotometric, HPTLC [15,16], Spectrofluorometric [17], Atomic absorption [18], Flow injection analysis [19,20], RP-HPLC [21]. Spectrophotometry [22-23] has been proposed for the determination of DPH and NAP in individual drug not in combination formulation. Many chromatographic methods such as gas chromatography [24], high

performance liquid chromatography (HPLC) have been used for the analysis of DPH and NAP in samples [25-28]. No much analytical method has been developed or reported for combination of these two drugs in pharmaceutical formulation. The HPLC method, which already published, is not determined on the tablet formulation, and in some article the retention time is too long when compared to the proposed method and the preparation of mobile phase is easy and cheaper when compared to published articles. The purpose of this study is to develop a new analytical method for the simultaneous estimation of DPH and NAP in raw and tablet formulation by RP-HPLC. In this proposed method a precise, simple, reproducible and specific method for estimation and determination of tablet form DPH and NAP simultaneously.

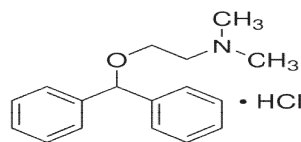


Fig. 1. Diphenhydramine HCl chemical structure

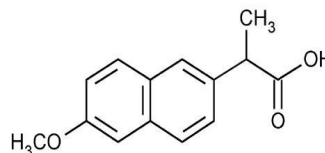


Fig. 2. Naproxen chemical structure

2. MATERIALS AND METHODS

2.1 Reagents

The 99.77% of DPH working standard and 99.72% NAP were obtained purchased from Sigma, UK. Combined tablet formulation of DPH HCl 5 mg and NAP 220 mg brand Aleve PM, Manufactured by Bayer Health Care Pharmaceuticals Inc, made in Germany.

Methanol and water (HPLC grade) were purchased from Merck, Darmstadt, Germany. Analytical grade Phosphoric acid, potassium dihydrogen phosphate and ortho phosphate used were purchased from Merck.

2.2 Preparation of Mobile Phase

The phosphate buffer was prepared using 0.51 g of KH_2PO_4 in 1000 ml of HPLC grade water, the pH adjusted to a 5.0 (± 0.5) by using 0.1 M solution of phosphoric acid. The resulting solution was filtered with 0.45 μ membrane filters and degassed in an ultrasonic bath for 15 mins. The ratio of Methanol: phosphate buffer was (30:70) v/v.

2.3 Chromatographic Conditions

A Shimadzu class LC-10A HPLC system equipped with LC – 10ATvp pump, SPD – 10A UV detector, and Rheodyne injector was used. Compounds were separated on a C18 hypersil (250 x 4.5 mm, 0.5 μ) column. The column temperature was maintained at a 27°C. The flow rate of 1.1 ml/min was set with Methanol and 0.5 mM phosphate buffer 30:70 v/v used as a mobile phase. The wavelength of 270 nm was set in detector. The peak responses area integrated using Shimadzu chromatographic software.

2.4 Preparation of Stock Solution (Standard)

1 mg of DPH-RS and 5 mg of NAP-RS taken separately in volumetric flask 50 ml and with mobile phase diluted to the mark. The mixture kept stand for 10 min, for complete solubility by intermittent sonication, and filtered through a 0.45 μ m membrane filter.

2.5 Working Standard Solution

4 ml of each stock solution (standard) from each were taken in 10 ml volumetric flask and diluted to 10 ml with mobile phase to get a concentration of 15 μ g / ml of DPH and 100 μ g/ml of NAP.

2.6 Sample Solution

Ten tablets of commercially available brand Aleve PM Tablets were taken and weighed and grinded into fine powder. 15 mg of powder equivalent was weighed and transferred into 25 ml volumetric flask then extracted with 25 ml of mobile phase. The resulting solution then filtered

by membrane filter 0.45 μ m and 1 ml of the aliquot was diluted again with 25 ml of mobile phase to achieve a concentration of 25 μ g/ml DPH and 220 μ g/ml NAP respectively.

2.7 Assay

20 μ l of sample and standard solutions were injected individually at different time point, into an HPLC injector; from the obtained HPLC peak areas of DPH and NAP amount of drug in sample were computed.

2.8 Method Validation

The present method was conducted to obtain a new, sensitive and convenient method for simultaneous estimation by HPLC. The experimental method was validated according to the ICH guidelines recommendations and USP-30 for parameters such as, system suitability, accuracy, precision, repeatability and specificity.

2.9 System Suitability

Suitability parameters like resolution, retention time, column theoretical plates and tailing factor was performed by injecting six replicates of standards and two replicates of sample preparation at a 100% level to cross verify the accuracy and precision of the chromatographic system.

2.10 Linearity

The linearity of chromatographic method was determined by plotting a graph to concentration vs peak area of DPH and NAP standard and determining the correlation coefficients (R^2) of the two compounds. For the linearity studies of DPH and NAP the specific range was determined at 15 – 35 μ g/ml and 100 – 500 μ g/ml for DPH and NAP respectively were injected into the HPLC system. The column was equilibrated with the mobile phase for 45 minutes before injection of the solutions.

2.11 Accuracy

The method accuracy was determined by recovery experiments. The experiment was performed by adding DPH and NAP working standards to placebo (excipients mixture) in the range of test concentration (60%, 80% and 100%) and expressed as percent (%) recovered. Three sets were prepared for each level

recovery. The recovery statistical results are under the acceptance range (S.D. < 2.0) value for DPH and NAP.

2.12 Precision

The precision of intraday and interday of the analyzed method was determined by 4 repeats of the sample responses on the same day and 4 different days of a week for 4 different concentrations of standard solutions of DPH and NAP. 15 – 35 µg/ml and 100 – 500 µg/ml for DPH and NAP respectively, and results are represented in terms of % RSD.

2.13 Specificity

The analytical method specificity is to measure the compound accurately in presence of interferences like excipients, degradants and matrix components. The RP-HPLC of standard mixture and formulation shows specificity of method. The RP-HPLC method is able to access the analyte in presence of excipients.

2.14 Robustness

For the robustness study the flow rate and wavelength detection were changed deliberate were made to evaluate the impact on the method and retention times were obtained for DPH and NAP respectively. The robustness of the method was assessed by altering the some experimental conditions such as, by changing the flow rate from 0.9 to 1.3 ml/min, amount of diluents (10% to 15%) the temperature of the column (25°C to 30°C).

2.15 Limit of Detection and Limit of Quantitation

Limit of detection and limit of quantitation represent the concentration of analyte that would yield signal to noise ratio of 3 for LOD and 10 for LOQ respectively. To determine LOQ and LOD serial dilutions of mixed standard solution of DPH and NAP was made from standard solution. The samples were injected in LC system and measured signal from the samples was compared with those of blank samples.

2.16 Statistical Parameters

The results of assay obtained are subjected to the following statistical analysis, standard deviation, relative standard deviation, coefficient of variation and standard error.

3. RESULTS AND DISCUSSION

According to USP-30 the system suitability test were tested on freshly prepared standard stock solution of DPH and NAP. The gradient elution with mobile phase A (Methanol and 0.5 mM phosphate buffer 30:70 v/v) and mobile phase B (Methanol and 0.5 mM phosphate buffer 40:60 v/v) gave the required separation of DPH and NAP. The gradient elution with combination of mobile phase A analysis revealed the HPLC method did not suffer from interference by the formulation excipients, there was no another peaks on the retention time of the drug. However, in the mobile phase B (Methanol and 0.5 mM phosphate buffer 40:60 v/v) the separation amongst peaks of the excipients from the formulation matrix could not be achieved. Therefore the composition of mobile phase A is fixed, as the high level of precision for the proposed method has been evidenced by the low values of standard deviation and standard error. Therefore, the optimum mobile phase of Methanol and 0.5 mM phosphate buffer 30:70 v/v ratios was selected. The ratios were found to resolve peaks ideally of DPH (3.1) and NAP (4.9) as the retention (chromatograph of test sample).

Several tests were performed for optimizing the components of mobile phase to achieve good chromatographic peak shape and resolution. Good separation of the target compounds and short run time were obtained by using a mobile phase system of methanol and 0.5 mM phosphate buffer (30:70, v/v). The retention time was found to be 3.1 minutes for Diphenhydramine and 4.9 minutes for Naproxen, were much shorter than those found in other papers (15, 18, 19, 21).

By scanning wide range of wavelength 200-400 nm wavelengths, the 270 nm was selected, DPH and NAP showed a good response at 270 nm. The Linearity was evaluated by plotting peak area as a functional of analyte concentration for both DPH and NAP. The graphical representation was given in (Figs. 3, 4).

The specific range was determined from linearity studies, for both drugs, which is 15-35 µg/ml for DPH and 100-500 µg/ml for NAP. The data was analyzed by linear regression least square fit method. The slop, intercept, correlation coefficient and regression equation were also determined and the data are presented in (Table 1).

The suitability parameters of the system like resolution, tailing factor, retention time and theoretical plates for the developed RP-HPLC method data are presented in (Table 2). The chromatographic retention time of DPH and NAP was found to be 3.1 and 4.9 minutes respectively. The chromatogram of the drug products was compared with the formulation and standard chromatogram presented in (Fig. 5). This is well within the specific limits of 10 minutes.

The tailing factor was found to be 1.27 and 1.16 for DPH and NAP respectively. The peaks are symmetrical and theoretical plates for DPH and NAP were 7216 and 9976 respectively, which shows the column efficient performance. The LOD and LOQ for DPH and NAP are presented in (Table 3). The quantitative estimation of the

tablet formulation is presented in (Table 4). The recovery study for spiked concentration of drugs to the pre analyzes form is represented in (Table 5). The robustness study for DPH and NAP is represented in (Table 6).

The tablet formulation assay shows percentage purity ranging from 99.19 to 100.92% for DPH and 99.51% to 100.21 for NAP. The percentage deviation was found to be -0.81 to +0.18% and -0.49 to +0.21 for DPH and NAP respectively. The RSD values are below 2% indicating the method precision and the accuracy of the method shown by the low standard error values. This shows a good index of accuracy and reproducibility of the developed method. All the parameters including flow rate, detection wavelength sensitivity was maintained constant.

Table 1. Results of statistical parameters

S. no	Parameters	Diphenhydramine	Naproxen
1	Standard deviation (SD)	4.749	5.773
2	Relative standard deviation (RSD)	0.01432	0.01259
3	% RSD	1.223	1.045
4	Standard error (SE)	0.0175	0.0195
5	Correlation coefficient (r)	0.9892	0.9997
6	Slope (a)	2040.7	2449.7
7	Intercept (b)	20465	32732
8	Regression equation $Y = (a X + b)$	$Y = 2040.7X - 20465$	$Y = 2449.7X - 32732$

Table 2. System suitability parameters

Parameter	Diphenhydramine	Naproxen
R T	3.1	4.9
Theoretical plates	7216	9976
Tailing factor	1.27	1.16
Resolution factor	3.7	5.2
Calibration range (or)	5 – 35 µg/ml	100 – 500 µg/ml
Linear dynamic range (LDR)		

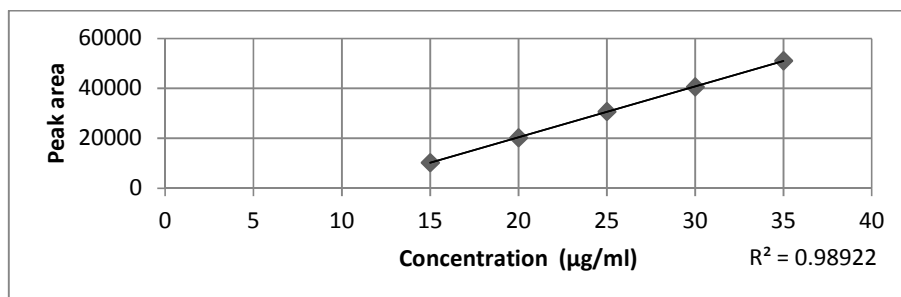


Fig. 3. Calibration curve of diphenhydramine

Table 3. Results of limit of detection (LOD) & limit of quantification LOQ

Parameters	Diphenhydramine	Naproxen
LOD (µg/ml)	2.21×10^{-4}	2.73×10^{-4}
LOQ (µg/ml)	7.2×10^{-4}	8.2×10^{-4}

Table 4. Quantitative estimation (assay) of data of diphenhydramine and naproxen

Diphenhydramine		Naproxen	
Amount claimed mg/tablet	Amount found mg/tablet	Amount claimed mg/tablet	Amount found mg/tablet
25	24.98	220	218.96
	25.01		219.68
	25.04		219.67
	24.92		220.52
	25.08		221.40
Mean	25.006	Mean	219.046

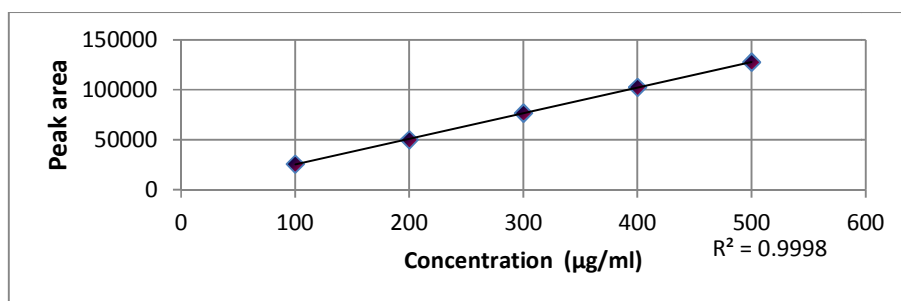
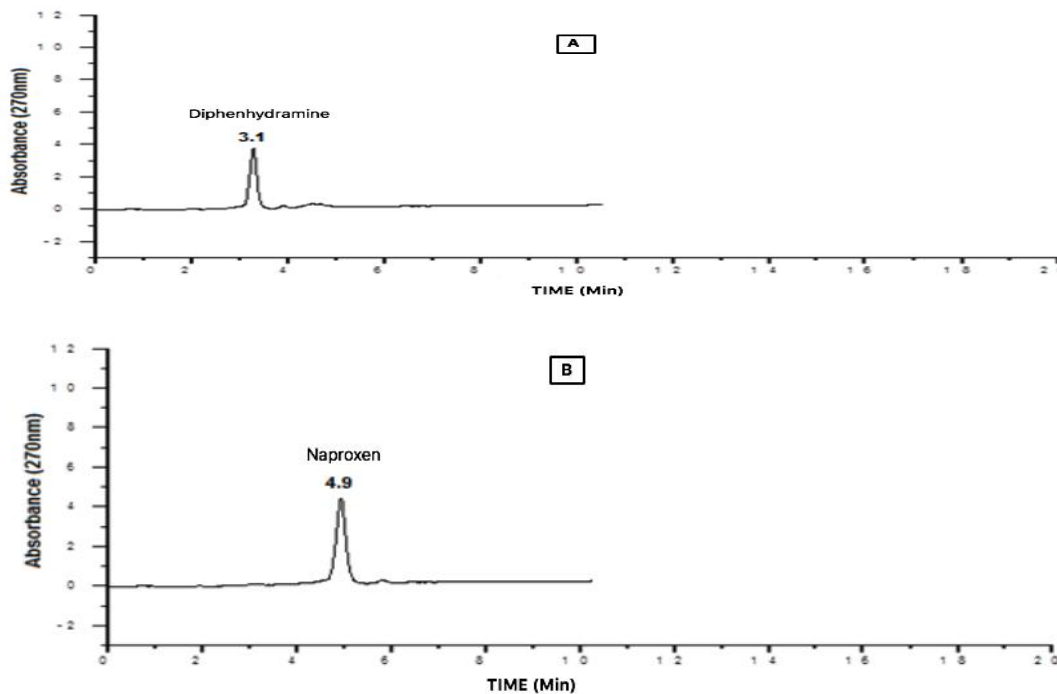


Fig. 4. Calibration curve of naproxen



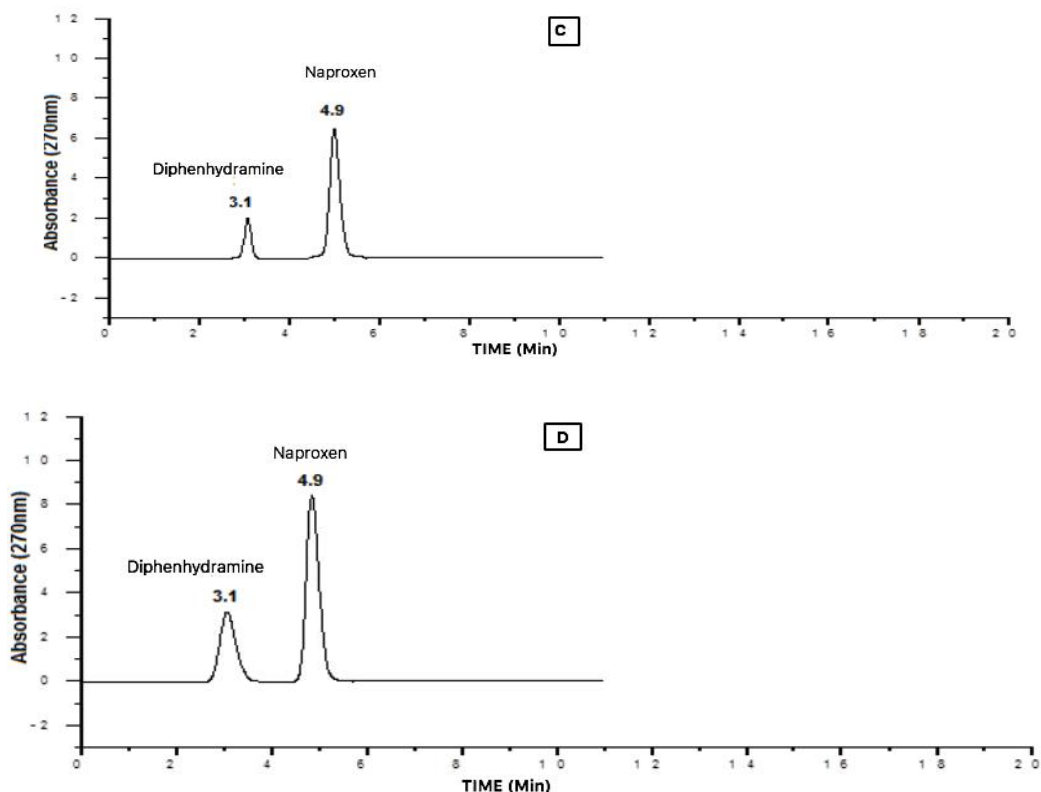


Fig. 5. The typical chromatogram of diphenhydramine shown in (A), Chromatogram of naproxen in (B), Chromatogram of diphenhydramine and naproxen in (C), Quantitative estimation of diphenhydramine and naproxen in tablet formulation in (D)

Table 5. Recovery study for spiked concentration of drugs to the pre analyzes form

Diphenhydramine			Naproxen		
Amount added (mg)	Amount found (mg)	Amount recovered (% mg)	Amount added (mg)	Amount found (mg)	Amount recovered (% mg)
25	24.98	99.74	220	121.42	101.11
35	35.07	100.72	240	239.52	99.41

Table 6. Robustness test of diphenhydramine and naproxen

Parameters	Changes	% recovery of diphenhydramine	% recovery of naproxen	% target
Flow rate (ml/min)	0.9	99.71	99.3	100%
	1.3	99.89	99.4	100%
Column temperature (°C)	25	99.7	99.5	100%
	30	99.6	99.5	100%

4. CONCLUSION

The proposed and developed RP-HPLC method is precise, accurate, and sensitive. The method is rapid, reproducible, and economical and does not have any interference due to the excipients in

the pharmaceutical preparations. The method shows good resolution time between DPH and NAP with short time (< 10 min). The proposed method is repeatable, very simple, and rapid involves no complicated sample preparation. High percentage of recovery result shows the

method is free from interference of excipients in the formulations.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Moffat AC, Osselton MD, Widdop B. Clarke's analysis of drugs and poisons, Pharmaceutical press. London. 2011;3: 1757-1777.
2. Goodman-Hillman A, Rall T, Nier A, Taylor P. The pharmacological basis of therapeutics, McGraw-Hill. New York; 1996.
3. Kolling WM, Ghosh TK. In theory and practice of contemporary pharmaceuticals. Jasti BR, Ghosh TK. Eds, 367, CRC Press Boca Raton; 2005.
4. Donmez OA, Asci B, Bozdogan A, Sungur S. Simultaneous determination of potassium guaiacol sulfonate, guaifenesin, diphenhydramine HCl and carbetapentane citrate in syrups by using HPLC-DAD coupled with partial least squares multivariate calibration. *Talanta*. 2011;83: 1601-1605.
5. Orkoula MG, Kontoyannis CG, Markopoulou CK, Koundourellis JE. Quantitative analysis of liquid formulations using FT-Raman spectroscopy and HPLC-The case of diphenhydramine hydrochloride in Benadryl. *Journal of Pharmaceutical and Biomedical Analysis*. 2006;41:1406-1411.
6. Tu RH, Grewall P, Leung JW, Suryaprasad AG, Sheykhzadeh PI, Doan C, Garcia JC. Diphenhydramine as an adjunct to sedation for colonoscopy: A double-blind randomized, placebo-controlled study. *Gastrointestinal Endoscopy*. 2006;63:87-94.
7. Hung CH, Chu CC, Chen YC, Chen YW, Li ZY, Wang JJ. Spinal anesthesia with diphenhydramine and pheniramine in rats. *European Journal of Pharmacology*. 2011; 67(3):20-24.
8. Martinez-Algaba C, Bermudez-Saldana JM, Villanueva-Camanas RM, Sagrado S, Medina-Hernandez MJ. Analysis of pharmaceutical preparations containing antihistamine drugs by micellar liquid chromatography. *Journal of Pharmaceutical and Biomedical Analysis*. 2006;40:312-321.
9. Luo H, Wang LJ, Wang J. Studies on quantitative determination of ingredients in dextromethorphan hydrobromide diphenhydramine hydrochloride paracetamol pseudoephedrine hydrochloride dispersed tablets by HPLC. *Yaowu Fenxi Zazhi Journal*. 2002;22:222-224.
10. Marchesini AF, Williner MR, Mantovani VE, Robles JC, Goicoechea HC. Simultaneous determination of naphazoline, diphenhydramine and phenylephrine in nasal solutions by capillary electrophoresis. *Journal of Pharmaceutical and Biomedical Analysis*. 2003;31:39-46.
11. European Pharmacopoeia. 5th ed. Council of Europe. Strasbourg; 2004.
12. Analgesics in: Wilson and Giswold's textbook of organic medicinal and pharmaceutical chemistry. 8th ed. New York; 2002.
13. Roberts Z, Morrow J. In Goodman & Gilman's the pharmacological basis of therapeutics, 10th ed. McGraw-Hill Medical Publishing Division, New York; 2001.
14. Hamilton M, Richart T, Tarascon. Pocket pharmacopoeia 2015 deluxe lab-coat edition. Jones & Bartlett Learning. 2015;9-14.
15. Dharmalingam S, Srinivasan R, Kumarappan C. Development and validation of UV spectrophotometric method for the estimation of naproxen in bulk and semi-solid formulation. *International Journal of Analytical. Pharmaceutical and Biomedical Sciences*. 2013;2:49-55.
16. Parekh SP, Dedania ZR, Dedania R, Vijayendraswamy SM. Analytical method development and validation of HPTLC method for simultaneous estimation of sumatriptan succinate and naproxen sodium in pharmaceutical dosage form. *International Journal of Ayurveda and Pharma Research*. 2014;2:94-99.
17. Ahmadi M, Madrakian T, Afkhami A. Enantio selective solid phase extraction prior to spectrofluorometric determination: A procedure for the determination of naproxen enantiomers in the presence of each other. *Royal Society of Chemistry Advances*. 2015;5:5450-5457.
18. El Ries MA and Khalil S. Indirect atomic absorption determination of atropine, diphenhydramine, tolazoline, and

- levamisole based on formation of ion-associates with potassium tetraiodometrcurate. *Journal of Pharmaceutical and Biomedical Analysis*. 2001;25:3-7.
19. Tipparat P, Lapanantnoppakhun S, Jakmunee J, Grudpan K. Determination of diphenhydramine hydrochloride in some single tertiary alkylamine pharmaceutical preparations by flow injection spectrophotometry. *Journal of Pharmaceutical and Biomedical Analysis*. 2002;30:105-112.
 20. Zhao C, Chai X, Tao S, Li M, Jiao K. Selective determination of diphenhydramine in compound pharmaceutical containing ephedrine by flow-injection electro chemiluminescence. *Analytical Science*. 2008;24:535-538.
 21. Amrohi S, Nasare M, Kumar T, Nivedita G, Diwan P. Analytical method development and validation for the estimation of Naproxen using RP-HPLC. *International Organization of Scientific Research*. 2012; 2:19-24.
 22. Khan I, Aman T, Ashraf A, Kazi A. Spectrophotometric determination of naproxen in pure and pharmaceutical preparations. *Analytical Letters*. 1999;32: 2035-2050.
 23. Mohammed AB. Visible spectrophotometric determination of chlorpheniramine maleate and diphenhydramine hydrochloride in raw and dosage form using Potassium permanganate. *Oriental Journal of Chemistry*. 2016;32:123-125.
 24. Kulsum S, Padmalatha M, Sandeep K, Saptasila B, Vidyasagar G. Spectrophotometric methods for the determination of naproxen sodium in pure and pharmaceutical dosage forms. *International Journal of Research in Pharmaceutical and Biomedical Sciences*. 2011;2:1303-1307.
 25. Raj SV, Kapadia SU, Argekar AP. Simultaneous determination of pseudoephedrine hydrochloride and diphenhydramine hydrochloride in cough syrup by gas chromatography. *Talanta*. 1998;46:221-225.
 26. Smith A, Jasim K, Varaprasad K. Development and validation of a stability indicating RP-HPLC method for analysis of naproxen sodium. *International Journal of Chemical Applications*. 2013;2:40-50.
 27. Tanjin S, Islam F, Sultan Z, Rahman A, Reza S, Sharmin T. Development and validation of a simple RP-HPLC method for determination of naproxen in pharmaceutical dosage forms. *Bangladesh Pharmaceutical Journal*. 2013;6:137-141.
 28. Toothaker RD, Barker SH, Gillen MV, Helsing SA, Kindberg CG, Hunt TL, Powell JH. Absence of pharmacokinetic interaction between orally co-administered naproxen sodium and diphenhydramine hydrochloride. *Bio Pharmaceutics and Drug Disposition*. 2000;21:229-233.

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