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Analytical Method Development and Validation for Simultaneous Estimation of Diphenhydramine Hydrochloride and Naproxen Sodium in its Combined Dosage Form

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ABSTRACT

A simple first order derivative spectrophotometric method was developed for simultaneous estimation of Diphenhydramine Hydrochloride (DPH) and Naproxen Sodium in combined dosage form. The analytical wavelengths selected for quantification were 247.4nm for NPS (zero crossing point for DPH) and 266nm for DPH (zero crossing point for NPS). Dual wavelength method was also developed for simultaneous estimation of DPH and NPS in combined dosage form. The analytical wavelength selected for quantification were 267.8 and 272.4nm and for DPH 247.6 and 249.6nm for NPS. The linearity was established over the concentration range of 15-35 µg/ml for DPH and NPS. The correlation coefficient (R^2) for DPH is 0.9993 and for NPS is 0.9991. The mean % recovery was found to be in range of 99.61% and 100.1%, DPH and NPS, respectively. The validation of the proposed method was found to be in compliance with the ICH guideline.

INTRODUCTION

Diphenhydramine hydrochloride (DPH) is chemically [2(diphenylmethoxy)ethyl]dimethylamine. DPH reduce the intensity of allergic symptoms. It is an antihistamine drug. DPH is used to treat sneezing, runny nose, watery eyes, hives, skin rash, motion sickness, to induce sleep, itching, etc. Chemical structure of DPH [1] was shown in Fig 1. It is official in IP[2] and USP[3].

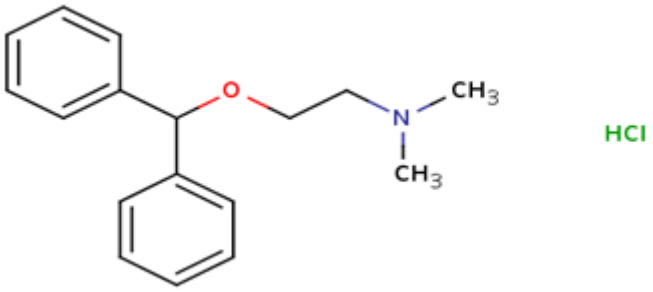

Figure1: Chemical Structure of Diphenhydramine Hydrochloride (DPH)

Naproxen Sodium (NPS) is chemically (2S)-2-(6-methoxynaphthalen-2-yl)propionic acid. NPS is a non-steroidal anti-inflammatory drug. NPS is used to treat pain or inflammation caused by condition such as arthritis, gout, bursitis, ankylosing spondylitis, etc. Chemical structure of NPS [4] was shown in Fig 2. It is official in BP and IP[5-6].

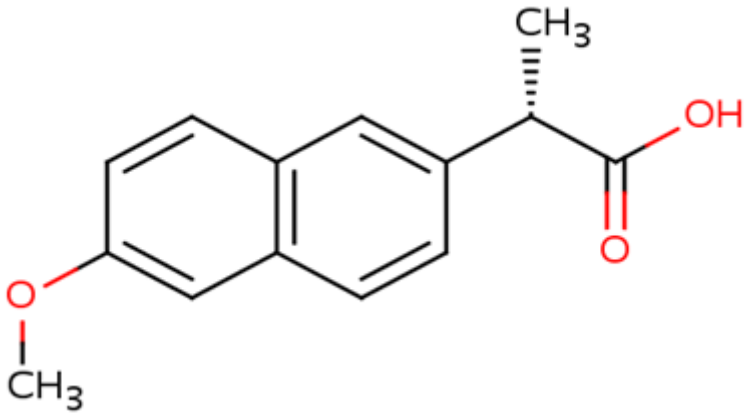

Figure 2: Chemical Structure of Naproxen Sodium(NPS)

Marketed tablet formulations of these agents play an important role in the treatment of occasional sleeplessness associated with minor aches and pains. The confirmation of the applicability of this developed method was validated according to the International Conference Harmonization (ICH) Q2 (R1)[7]. A thorough literature survey was carried out and revealing that

many analytical methods like HPLC[8-12], UV[13,14], HPTLC[15-17], RP-UPLC[18,19] were developed for the estimation of DPH and NPS individually as well in combination with other dosage form. But no first order derivative spectrophotometric method was developed for estimation of these two in its combined dosage form.

MATERIALS AND METHODS

Experimental Work

Materials and Reagents

Pure drug sample of NPS was gifted by RPG Life Sciences Limited and pure sample of DPH was gifted by Mahrshee Laboratories PVT. LTD. The gifted sample was used as standard without any further purification. Distilled grade water was used as solvent for both drugs.

Instrumentation

Shimadzu UV-1700 a double beam spectrophotometer, connected to a computer loaded with Shimadzu UV probe 2.34 software was used for all the spectrophotometric measurements. The absorbance spectra of the test solutions were carried out in 1cm quartz cells over the range of 200-400nm.

Preparation of standard stock solution

A 100 mg of DPH and NPS standard were weighed accurately separately and transferred to a 100 ml volumetric flask and dissolved in water to give a solution containing 1000 µg/ml DPH and NPS respectively. 100 µg/ml of DPH and NPS stock solution was prepared by diluting 10.0 ml stock solution to 100 ml with water separately.

Selection of solvent (For First Order Derivative and For Dual Wavelength)

Both the drugs are soluble in distilled water. The overlain spectra of DPH and NPS, when overlapped, shows feasibility of using this solvent for spectrophotometric analysis for simultaneous estimation of these drugs. Therefore, distilled water was selected as solvent.

Selection of wavelength and preparation of calibration curve

For First Order Derivative

Solution of DPH and NPS were recorded in the range of 200-400nm. All zero order spectra (D0) converted to 1st order derivative spectrum (D1). The 1 st order derivative of the standard solution was traced with scaling factor (factor 10) and delta lambda 4. Absorbance at 247.4nm (zero crossing point of DPH) was plotted against the concentration of NPS. Similarly, the absorbance at 266nm (zero crossing point of NPS) was plotted against the concentration of DPH to construct two separate calibration curves for both the drugs. Method showed good linearity in concentration range of 15-35µg/ml for both the drugs.

For Dual Wavelength

The absorption spectra of solutions of DPH and NPS were recorded in the range of 200-400nm and zero order spectra was taken. Then Dual wavelength method was applied. Absorbance difference of DPH at λ= 267.8 and 272.4nm where taken for plotting CC of DPH were the same for NPS is 0. Similarly, absorbance difference of NPS at λ= 247.6 and 249.6nm where taken for plotting CC of NPS were the same for DPH is 0. The range of both the drug was found to be 15 – 35µg/ml.

METHOD VALIDATION

Linearity and range

The linearity response was determined by analyzing independent levels of concentrations in the range of 15-35 µg/ml for DPH and NPS respectively. Absorbance of each solution was measured at 266 and 247.4nm were recorded for DPH and NPS respectively for 1st order derivative. Absorbance of each solution was measured at 267.8-272.4 and 247.6-249.6nm for dual wavelength. Calibration curve was constructed by plotting absorbance versus concentration for both drugs. The correlation coefficient and regression line equations for DPH and NPS were determined.

Precision

Repeatability

For repeatability, six replicates of standard mixture solution having DPH and NPS (25µg/ml) were prepared and absorbance were recorded at 266 and 247.4nm were recorded for DPH and NPS respectively for 1st order derivative. And absorbance was recorded at 267.8-272.4 and 247.6-249.6nm respectively for Dual wavelength. SD and RSD were calculated.

Intraday and Interday Precision

Intraday and Interday precision study of DPH and NPS was carried out by estimating different concentrations of DPH and NPS (15, 25,35 µg/ml), three times on the same day and on three different days and the results are reported in terms of % RSD. The limit for %RSD should be NMT 2%.

Accuracy (Recovery Study)

Accuracy was determined by performing recovery studies by spiking specific concentration of marketed formulation at 3 levels (80%, 100%, 120%) in triplicate to pre-analyzed sample solution of 15µg/ml of DPH and NPS. Pre-analyzed sample was added which was at different level 80, 10 and 120%. Each concentration was analysed 3 times and average recoveries were measured.

LOD and LOQ

Calibration curve was repeated 6 times and standard deviation of intercept and average of slope was calculated. Then LOD and LOQ were measured as follows.

LOD= 3.3*SD/Slope of calibration curve
LOQ= 10*SD/Slope of calibration curve
SD= Standard deviation of intercepts

Specificity

Specificity was determined by performing studies by spiking specific concentration of excipient in pre-analyzed sample solution of 15µg/ml of DPH and NPS. To preanalyzed sample solution, a

known amount of excipient stock solution was added which was at different level 75, 100 and 125%. The solution was analyzed by proposed method. Mean % recovery was calculated.

Assay

20 tablets of aleve.pm (Marketed Formulation) were weighed accurately and average weight (1.06gm) was taken. Then all the 20 tablets were crushed and equivalent powder (0.070gm) was taken and volume was made up with water up to 200ml. From above stock 10ml was pipetted out and again volume was made up to 100ml and standard of DPH of 31µg/ml was added to the solution and measured in the UV.

RESULTS AND DISCUSSION of UV First Order Derivative Spectroscopy Method

Table 1. Regression analysis data for 1st order derivative of DPH and NPS

PARAMETERS	DPH (266 nm)	NPS (247.4 nm)
Conc. Range (µg/ml)	15-35	15-35
Regression equation (y = mx + c)	0.0014x+0.0042	0.0069x+0.0605
Correlation Coefficient(r^2)	0.999	0.999
Slope (m)	0.0014	0.0069
Intercept (c)	0.0042	0.0605

Table 2. Regression analysis data for Dual Wavelength of DPH and NPS

PARAMETERS	DPH (267.8 - 272.4 nm)	NPS (247.6 - 249.6 nm)
Conc. Range (µg/ml)	15-35	15-35
Regression equation (y = mx + c)	0.0006x - 0.0003	0.0006x + 0.0086
Correlation Coefficient(r^2)	0.999	0.999
Slope (m)	0.0006	0.0006
Intercept (c)	0.0003	0.0086

Table 3. % Recovery of DPH and NPS

Analyte	Amount of std	Amount recovered	% Recovery	Mean recovery ± SD	Overall (mean±%RSD)
NPS	12	11.83	99.38	98.55 ± 0.0084	99.25 ±0.24
	15	29.69	98.97	98.47± 0.042	
	18	39.77	99.42	99.39 ± 0.038	
DPH	12	19.98	98.49	98.49 ± 1.54	99.60 ±0.43
	15	29.73	100.36	100.36 ± 1.23	
	18	39.92	99.79	98.53 ± 1.78	

Table 4. Intraday and Interday DPH and NPS

Type of precision	Conc. (µg/ml) DPH: NPS	Average peak area (mV*s)		% RSD	
		DPH	NPS	DPH	NPS
Intraday (n=3)	15:15	14.76 ± 0.23	15.09 ± 0.23	1.59	1.49
	25:25	25.08 ± 0.36	24.98 ± 0.42	1.44	1.66
	35:35	35.08 ± 0.46	35.23 ± 0.58	1.33	1.66
Interday (n=3)	15:15	15.07± 0.22	14.89± 0.22	1.48	1.50
	25:25	24.80± 0.12	25.00± 0.17	0.49	0.70
	35:35	34.80± 0.27	34.84± 0.21	0.78	0.62

Table 5. Specificity of DPH and NPS

Analyte	% Spike	Preanalysed	Spike conc	Absorbance	Found conc	% Found
DPH	75	15	11	0.0413	26.16	100.63
	100	15	15	0.0464	29.77	99.29
	125	15	18	0.0512	33.15	100.48
NPS	75	15	11	0.2350	25.77	99.16
	100	15	15	0.2630	29.50	98.36
	125	15	18	0.2844	32.42	98.26

Table 6. LOD and LOQ DPH and NPS

Parameters	DPH	NPS
SD of Intercept	0.00418	0.04172
Slope	0.00142	0.0069
LOD (µg/ml)	1.61	1.99
LOQ (µg/ml)	4.90	6.04

Table 7. Assay of DPH and NPS

Drugs	Conc. in dosage form	Conc. Found (µg/ml) ± SD (n=5)	Assay ± SD	%RSD
DPH	35	34.57 ± 0.39	99 ± 1.11	1.13
NPS	35	34.57 ± 0.32	99 ± 0.92	0.93

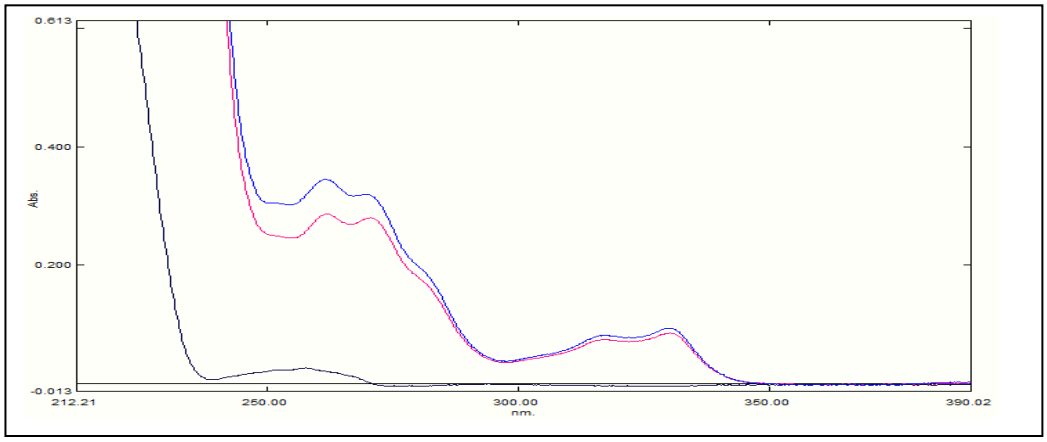

Figure 3: Overlay of NPS, DPH and MIX

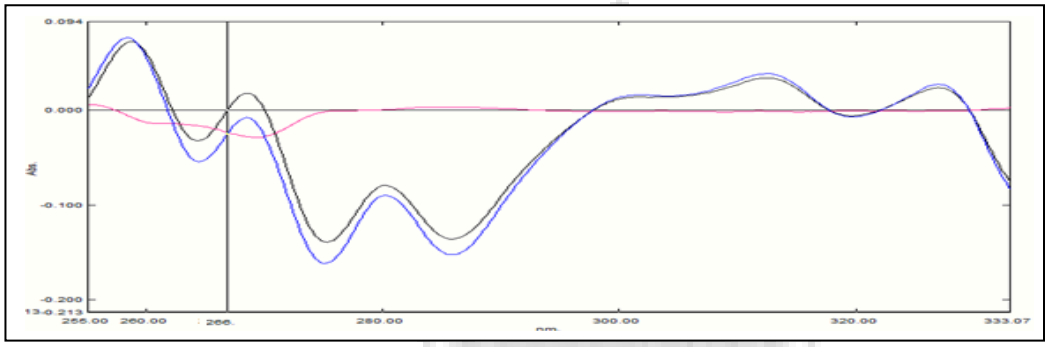

Figure 4: First order derivative spectra of DPH, NPS and MIX at 266nm.

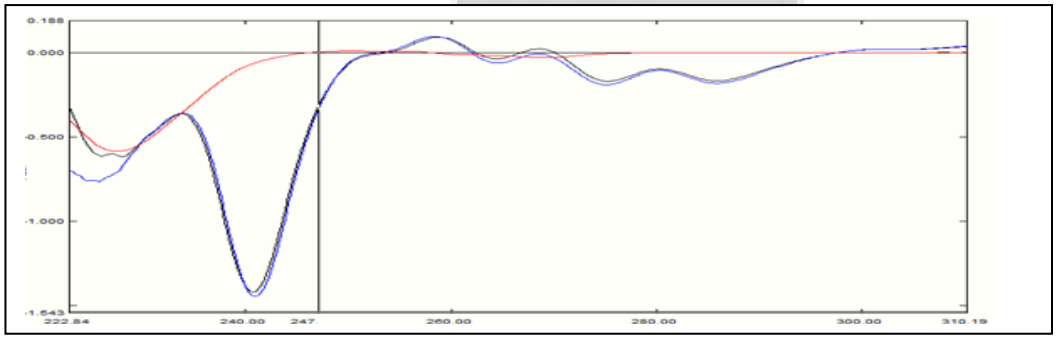

Figure 5: First order derivative spectra of DPH, NPS and MIX at 247nm.

CONCLUSION

All the methods were found to be simple, accurate and reproducible. The methods were validated as per ICH guidelines. The methods can be successfully applied for routine QC analysis.

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