International Journal of Trend in Scientific Research and Development (IJTSRD)
Volume: 3 | Issue: 2 | Jan-Feb 2019 Available Online: www.ijtsrd.com e-ISSN: 2456 - 6470

Multiple Method Development and Validation for Simultaneous Estimation of Chlorzoxazone and Nimesulide in Bulk and Pharmaceutical Dosage Form

Swetha Yarramsetti, A. Elphine Prabahar, Rama Rao Nadendla

Department of Pharmaceutical Analysis, Chalapathi Institute of Pharmaceutical Sciences, Lam, Guntur, Andhra Pradesh, India

ABSTRACT

A simple, precise and accurate multiple analytical method has been developed for the simultaneous estimation of Chlorzoxazone and Nimesulide in bulk and tablet formulations by reversed-phase liquid chromatographic and UV-Visible spectrophotometric techniques. The chromatographic separation was achieved on C18 analytical column. A mixture of Methanol: 0.1% Ortho-phosphoric acid (75:25) was used as mobile phase, at a flow rate of 1mL/min and detection wavelength at 295 nm. The retention time of Chlorzoxazone and Nimesulide was found to be 4.69 and 5.45 min respectively. The linear dynamic ranges for HPLC were from 2-10 µg/mL and for simultaneous equation method, derivative spectroscopy, Q-ratio Absorbance method, Dual wavelength it was 10-30 µg/mL for both Chlorzoxazone and Nimesulide. The percentage recovery obtained for Chlorzoxazone and Nimesulide were 100.93 and 102.19 % respectively for RP-HPLC, 9.7 % and 100.1 % for simultaneous equation method of CZ and NIM respectively, 99.97 % and 99.78 % for derivative spectroscopy of CZ and NIM respectively, 101.37 % and 99.48 % for Q-ratio Absorbance method of CZ and NIM respectively, 100.13 % and 99.96 % for dual wavelength method of CZ and NIM respectively. The validation of the proposed methods were carried out for linearity, accuracy, precision, limit of detection, limit of quantitation and robustness. The developed method can be used for routine quality control analysis of titled drugs in combination in tablet formulation.

Keywords: *Chlorzoxazone, Nimesulide, RP-HPLC, Uv-spectroscopy, derivative spectroscopy, Q-ratio Absorbance, Dual wavelength*

INTRODUCTION

Chlorzoxazone is a centrally acting central muscle relaxant with sedative properties. It is 5-chloro-2,3-dihydro-1,3-benzoxazol-2-one, which act by inhibiting calcium and potassium influx which would lead to neuronal inhibition and muscle relaxation. Nimesulide is a relatively COX-2 selective, non-steroidal anti-inflammatory drug (NSAID) with analgesic andantipyretic properties. Chemically it is N-(4-nitro-2-phenoxyphenyl) methane sulphonamide.

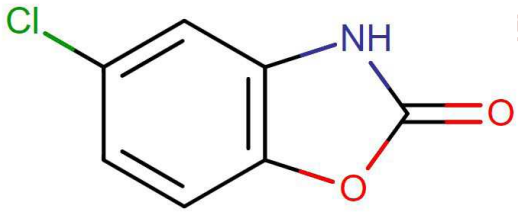

Figure: 1 Structure of Chlorzoxazone

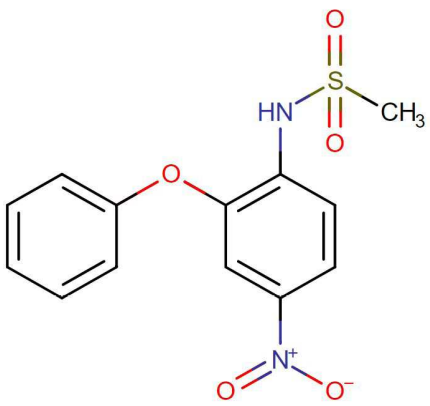

Figure: 2 Structure of Nimesulide

In this paper we have reported some of the analytical methods for the simultaneous determination of Chlorzoxazone and Nimesulide. Literature survey reveals only RP-HPLC and UV-spectroscopy for the simultaneous determination of the drugs in bulk or in the combined dosage forms and no spectroscopic methods such as Absorbance Ratio Method, Dual Wavelength Method and Derivative Spectroscopic Method. The method is described as new, simple, fast, rapid, accurate, efficient, reproducible for the development and validation analysis of simultaneous estimation of Chlorzoxazone and Nimesulide in pharmaceutical dosage form as per ICH guildelines.

EXPERIMENTAL SECTION

Chemicals and reagents used:

The reference standards of Chlorzoxazone and Nimesulide was procured from Aurbindo Pharma Limited, Hyderabad, India. Chemicals such as Acetonitrile, Methanol, Ortho-phosphoric acid and Dipotassium hydrogen Phosphate that were used for the chromatographic procedures are HPLC grade of Merck. Water for HPLC was used for the preparation of mobile phase. Pharmaceutical dosage form manufactured by Gracure Pharmaceutical Limited, (brand name- Nimox) by the composition of Nimesulide-100 mg and Chlorzoxazone-250 mg was used for the study.

Instrumentation:

LABINDIA-UV 3092 UV/VIS spectrophotometer and HPLC system (AGILENT HPLC Model-1220 Infinity-LC with Ezchromelite Software) with gradient elution containing C 18 column with UV-detector were used.

Chromatographic conditions:
The column used for chromatographic separations was C 18 (4.6 i.d., 250 mm length, 5 µm particle size). The analytical wavelength was set at 295 nm and samples of 20µl was injected manually. Chromatographic separations were performed by the mobile phase composition of Methanol : 0.1% Ortho-phosphoric acid (75:25) which is filtered through 0.22 µm membrane filters and degassed in the ultrasonic bath. Mobile phase was injected through isocratic conditions with the flow rate of about 1ml/min.

Preparation of standard and sample solutions:
The standard solutions of Chlorzoxazone and Nimesulide was prepared by dissolving 10mg of drug in 3ml and 10ml of HPLC grade methanol respectively and the volume was made upto 10ml with HPLC grade water. Linear dynamic ranges for HPLC was prepared from 2-10 µg/mL by the standard solutions.

Concentration ranges of about 10-30 µg/mL for both Chlorzoxazone and Nimesulide was prepared with distilled water and scanned at the UV range against blank solution for simultaneous equation method, derivative spectroscopy, Q-Ratio Absorbance and Dual wavelength methods.

For the sample preparation 10 tablets were taken pulverized and weight equivalent to 10mg was taken and dissolved in 10ml of HPLC grade methanol. The solution was further diluted with HPLC grade water to get the concentration of 8µg/ml.

RESULTS AND DISCUSSION
FOR HPLC:
The standard solutions of both Chlorzoxazone and Nimesulide were scanned in the UV range of 200-400 nm against the blank solution and the wavelength of 295 nm was found to show appreciable absorbance for both the drugs.

Many trails were performed by the change of different columns, mobile phase and their ratios out of which a suitable method was developed by the C18 column, mobile phase of Methanol: 0.1% Ortho-phosphoric acid (75:25), with the flow rate of about 1 ml/min and the run time of 7min. Peaks were eluted and showed better resolution, theoretical plate count and asymmetry was found at 4.69 and 5.45 min respectively for Chlorzoxazone and Nimesulide. The chromatogram of both the drugs was shown in the Figure 3.

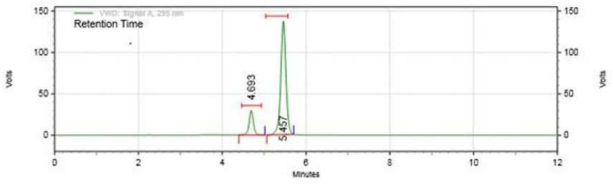

Figure 3: Chromatogram of Chlorzoxazone and Nimesulide.

FOR SIMULTANEOUS EQUATION METHOD:
The standard solutions of both Chlorzoxazone and Nimesulide were scanned in the UV range and the wavelength was found to be 280 nm and 390 nm respectively. Calibration curves were plotted for both the drugs with the concentration range of 10-30 µg/mL. Absorption spectrum for both the drugs was shown in the Figure 4 and Figure 5 respectively. The absorbance and absorptivity values at particular wavelengths were submitted in the following equation to obtain the concentration.

Cx = (A2ay1-A1ay2)/ (ax2ay1-ax1ay2)

Cy = (A1ax2-A2ax1)/ (ax2ay1-ax1ay2)

Where,

Cx = concentration of CZ

Cy = concentration of NIM

A1 = absorbance of samples at 280 nm.

A2 = absorbance of samples at 390 nm.

ax1 is the absorptivity of CZ at 280 nm.

ax2 is the absorptivity of CZ at 390 nm.

ay1 is the absorptivity of NIM at 280 nm.

ay2 is the absorptivity of NIM at 390 nm.

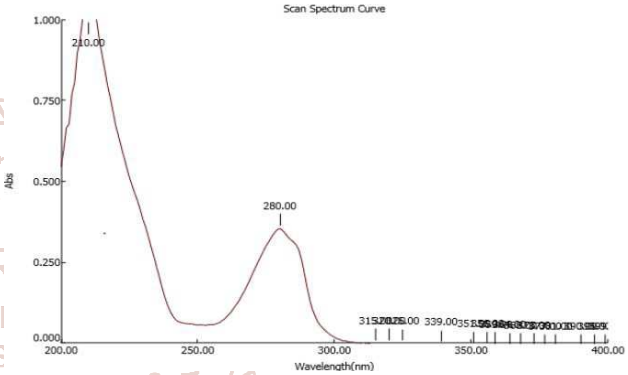

Figure 4: Spectrum of Chlorzoxazone at 280 nm.

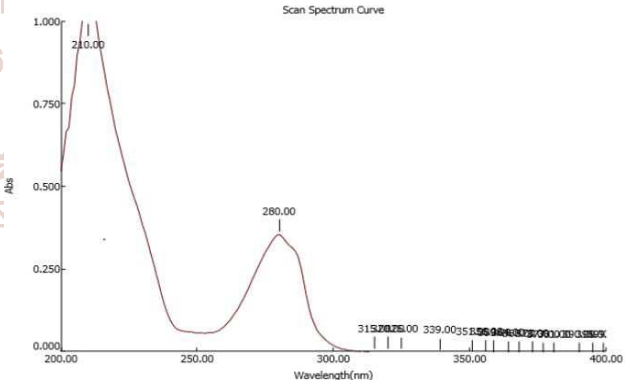

Figure 5: Spectrum of Nimesulide at 390 nm.

FOR SPECTROPHOTOMETRIC FIRST DERIVATIVE ZERO-CROSSING METHOD:
Working standard stock solutions were scanned in the UV range (215-400 nm), the absorption spectra thus obtained were derivatized to first order and from the overlain spectra of both drugs wavelengths were selected.

First order derivative spectra was determined for both drugs as shown in the Figure 6-7. zero crossing point was at 244 nm and 388 nm for Chlorzoxazone and nimesulide respectively.From the overlain spectra of both the drugs wavelengths selected for quantization were 388nm for CZ (Zero crossing point of NIM) and 244 nm for NIM (Zero crossing point of CZ). The calibration curves for CZ and NIM was plotted in the concentration range of 10-30µg/ml at wavelengths of 244 nm and 388 nm respectively.

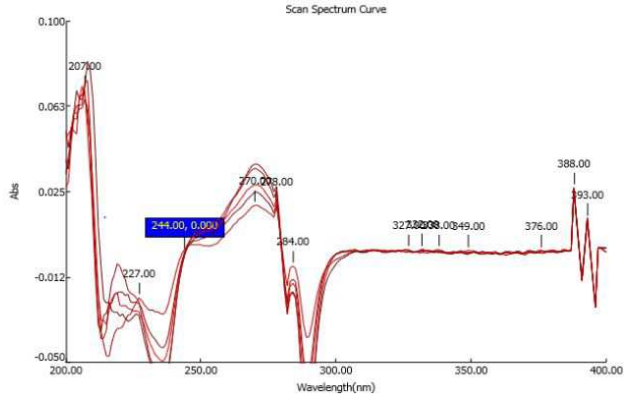

Figure 6: first order derivative overlain spectra of Chlorzoxazone at 244 nm.

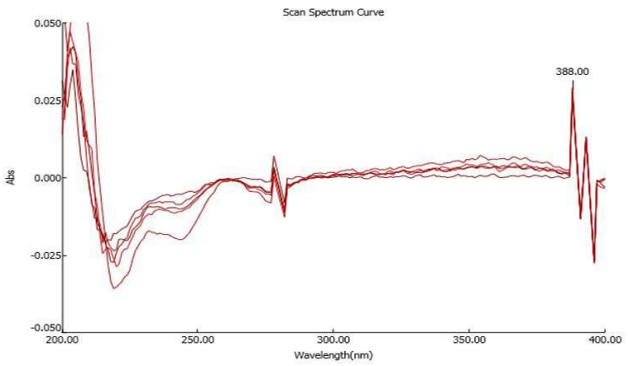

Figure 7: first order derivative overlain spectra of Nimesulide at 388 nm.

FOR Q-RATIO ABSORBANCE METHOD:

From the overlain spectrum of both the drugs isoabsorptive point for the drugs was found to be 268 nm.The concentration of two drugs in the mixture was calculated by using following equations.

$CX = (QM - QY)*A1 / (QX - QY)* aX1$

$CY = A1/ aX1 - CX$

Where A1 and A2 are absorbance's of the mixture at 280nm and 390nm respectively; aX1 and aY1 are absorptivites of CZ and NIM respectively at 280nm and aX2 and aY2 are absorptivites of CZ and NIM respectively at 390nm.

QM = A2/A1, QX = AX2/AX1 and QY = AY2/AY1.

Overlain spectrum of the drugs was shown in the Figure 8.

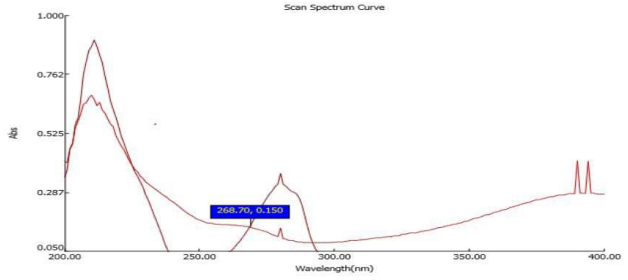

Figure 8: Overlain spectra of CZ and NIM showing Iso-Absorptive point at 268nm

FOR DUAL WAVELENGTH METHOD:

10µg/ml concentration of both the drugs were scanned in the UV range and from the overlain spectra of both the drugs 279nm and 289nm was selected as λ1 and λ2 for estimation of CZ where NIM shows the same absorbance at this wavelength. Similarly, wavelengths 386nm and 398nm were selected as λ1 and λ2 for estimation of NIM where CZ shows the same absorbance at these wavelengths. Overlain spectrum for dual wavelength method was shown in the Figure 9.

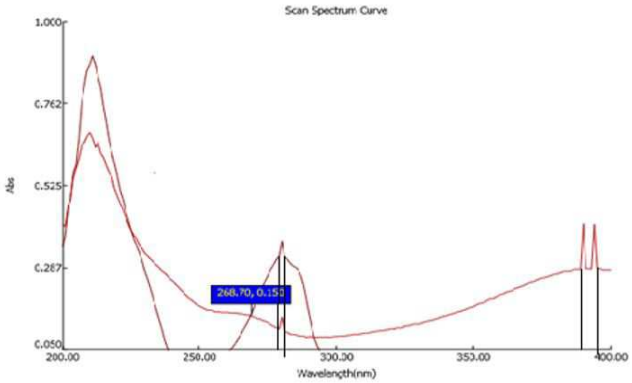

Figure 9: Spectrum of Dual wavelength method

METHOD VALIDATION:

LINEARITY:

The Linearity of analytical method is its ability to obtain test results, which are directly proportional to the concentration of analyte in the test sample. It is evaluated by analyzing a series of different concentrations ranging between 2-10 µg/ml and 10-30 µg/ml of both CZ and NIM for HPLC and other methods respectively. Calibration graphs were plotted and linear relationship was established.

PRECISION:

The precision of the analytical method was studied by analysis of multiple sampling of homogenous sample, expressed as %RSD. Method Reproducibility was demonstrated by repeatability and intermediate precision measurements. The repeatability (within in the day) and intermediate precision (for 3 days) was carried for each compound. The obtained results within and between days trails are in acceptable range indicating good precision of the proposed method.

ACCURACY:

The accuracy of an analytical method is the closeness of test results obtained by that method compared with the true values. To confirm the accuracy of the proposed method, recovery experiments were carried out by standard addition technique. The accuracy of the method was carried out by adding by known amounts of each drug corresponding to three concentration levels, 80, 100 and 120% of the label claim along with the excipients in triplicate. The samples were given the same treatment as described in sample preparation. The percentage recoveries of CZ and NIM at each level and each replicate were determined. The mean of percentage recoveries (n=3) and the relative standard deviation was calculated. It was confirmed from results that the method is highly accurate.

LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION:

Calibration curve was repeated for 5 times and the standard deviation (SD) of the intercepts was calculated for UV and Chromatogram signals obtained with known low concentrations of analytes was compared with the signals of blank samples, a signal- to- noise ratio 3:1 and 10:1 is considered for calculating LOD and LOQ respectively for HPLC.

ROBUSTNESS:
The robustness of the method was determined by making slight changes in the chromatographic conditions. The robustness of the proposed HPLC method was assessed for peak resolution and symmetric factor. The parameters investigated are Apparent pH of the mobile phase, Mobile phase organic content, Mobile phase flow rate, Detection wave length.

SYSTEM SUITABILITY:
System precision was determined on six replicate injections of standard preparation all important characteristics including %RSD, resolution (between CZ and NIM), tailing factor and theoretical plate number were measured.

ASSAY OF TABLETS:
Applicability of the method was tested by analyzing the commercially available formulations containing the binary mixture of CZ and NIM. The values of % recovery from formulations are found to be very close to each other as well as to the label value of commercial pharmaceutical formulation.

Results for the validation parameters for all the methods are shown in Tables

Table 1: Simultaneous estimation of Chlorzoxazone and Nimesulide by HPLC

Parameters	Chlorzoxazone	Nimesulide
Linearity	2-10µg/mL	2-10µg/mL
Equation Y=mx	Y = 1E+06x	Y = 229436x
Correlation coefficient	0.9914	0.9948
LOD (µg/mL)	0.633	0.304
LOQ (µg/mL)	1.90	0.912
Precision Intraday	0.346	1.66
Precision Interday	0.403	1.93
Theoretical plates	9880	10773
Asymmetry	0.99	1.02
Retention time	5.467	4.720
% Recovery	100.93	102.19
Amount found in formulation(mg)	249.5	102.09

Table 2: Simultaneous estimation of Chlorzoxazone and Nimesulide using simultaneous equation method

Parameters	Chlorzoxazone (280 nm)	Nimesulide (390 nm)
Linearity	10-30µg/mL	10-30µg/mL
Equation (y=mx)	y = 0.025x	y = 0.024x
Correlation coefficient	0.996	0.998
LOD (µg/mL)	3.33	3.57
LOQ (µg/mL)	10	10.71
Precision Intraday	0.561	0.389
Precision Interday	0.524	0.457
% Recovery	99.7	100.01
Amount found in formulation(mg)	248.89	99.23

Table 3: Spectrophotometric first derivative Zero-crossing method for simultaneous estimation of Chlorzoxazone and Nimesulide

Parameters	Chlorzoxazone (244 nm)	Nimesulide (388 nm)
Linearity	10-30µg/mL	10-30µg/mL
Equation (y=mx)	y = 0.026x	y = 0.0207x
Correlation coefficient	0.993	0.9913
LOD (µg/mL)	3.95	3.66
LOQ (µg/mL)	10.94	9.98
Precision Intraday	0.4315	0.441
Precision Interday	0.430	0.853
% Recovery	99.97	99.78
Amount found in formulation(mg)	250.14	99.89

Table 4: Q-Absorbance Ratio method for the simultaneous estimation of Chlorzoxazone and Nimesulide

Parameters	Chlorzoxazone (268 nm)	Nimesulide (268 nm)
Linearity	10-30µg/mL	10-30µg/mL
Equation (y=mx)	y = 0.0298x	y = 0.0029x
Correlation coefficient	0.992	0.997
LOD (µg/mL)	3.75	3.66
LOQ (µg/mL)	10.25	9.98
Precision Intraday	0.361	1.67
Precision Interday	0.540	1.745
% Recovery	101.37	99.48
Amount found in formulation(mg)	250.15	99.85

Table 5: Dual wavelength method for simultaneous estimation of Chlorzoxazone and Nimesulide

Parameters	Chlorzoxazone	Nimesulide
Linearity	10-30µg/mL	10-30 µg/mL
Equation (y=mx)	y = 0.0003x	y = 0.0028x
Correlation coefficient	0.9924	0.997
LOD (µg/mL)	3.41	3.63
LOQ (µg/mL)	10.23	9.89
Precision Intraday	1.96	1.696
Precision Interday	1.258	1.66
% Recovery	100.13	99.96
Amount found in formulation (mg)	248.98	99.63

CONCLUSION
All the proposed spectrophotometric and chromatographic methods were found to be simple, sensitive, accurate and precise for the determination of Chlorzoxazone and Nimesulide in the pharmaceutical dosage form. The methods utilizes easily available and less economical solvent for analysis of Chlorzoxazone and Nimesulide hence the methods were economical for the estimation of Chlorzoxazone and Nimesulide in synthetic mixture.

REFERENCES

[1] Snehal J. More, Suparna S. Tandulwadkar, Ajinkya R.Nikam, AtulS.Rathore, L.Sathiyanarayanan, and Kakasaheb R.Mahadik (2012). Application of HPLC for the Simultaneous Determination of Paracetamol, Chlorzoxazone, and Nimesulide in Pharmaceutical Dosage Form. ISRN Chromatography Volume 2012, Article ID: 252895, P1-8.

[2] S. Ravisankar, M. Vasudevan, M. Gandhimathi, B.Suresh. (1998). Reversed-phase HPLC method for the estimation of acetaminophen, ibuprofen and chlorzoxazone in formulations. Elsevier, 46(6), P 1577-1581.

[3] Vinod Maheshwari, Jaydeep Sangani, Naimish Kariya, Payal Maheshwari, Jenisha Roka. Development And Validation Of Rp-Hplc Method For Simultaneous Estimation Of Diclofenac Sodium And Chlorzoxazone In Their Combined Tablet Dosage Form. Inventi: ppaqa/1049/13.

[4] Jigar Patel, Pinak Patel (2014). Rp-Hplc Method Development And Validation For The Estimation Of Diclofenac Sodium, Tramadol Hydrochloride And Chlorzoxazone From Their Combined Tablet Dosage Form. International Journal of Pharmacy and Pharmaceutical Sciences. 6(7), P632-637.

[5] Shaikh K, Devkhile A (2008). Simultaneous determination of aceclofenac, paracetamol, and chlorzoxazone by RP-HPLC in pharmaceutical dosage form. J Chromatogr Sci. 46(7), P649-52.

[6] Nitin D. Rawool*, A. Venkatchalam (2013). Development and validation of a rapid HPLC method for the simultaneous estimation of nimesulide and tizanidine hydrochloride in pharmaceutical tablet dosage form. ACAIJ, 12(11), P408-414.

[7] British Pharmacopoeia 2007, Volume I & II, Accessed soft copy.

[8] Indian Pharmacopoeia 2007, Volume II,Published by the controller of Publication, Ministry of Health and Family welfare, New Delhi 681.

[9] Yousry M. Issa, Sayed I.M. Zayed, Ibrahim H.I. Habib (2010). Simultaneous determination of ibuprofen and paracetamol using derivatives of the ratio spectra method. Arabian Journal of Chemistry. 4, P259-263.

[10] Dr. A. Rajasekaran (2010). Hand book of ultra-visible & infrared spectroscopy. Tamil Nadu: Rupi publication. P76-83.