World Journal of Pharmaceutical research

Volume 1, Issue 5, 1361-1370. Research Article ISSN 2277 – 7105

# DEVELOPMENT OF SPECTROPHOTOMETIC METHODS FOR THE ESTIMATION OF WATER INSOLUBLE CALCIUM CHANNEL BLOCKERS USING HYDROTOPIC SOLUBILIZATION TECHNIQUE

***Naresh Kalra, Suresh Choudhary**

Department of Pharmaceutical sciences, Alwar Pharmacy College, IET, North Extension M.I.A, Alwar, India.

Article Received on
05 October 2012,

Revised on 17 October 2012,
Accepted on 26 October 2012

***Correspondence for Author:**

*** Naresh Kalra**

Department of Pharmaceutical sciences, Alwar Pharmacy College, IET, North Extension M.I.A, Alwar, India

neshu07@gmail.com

## ABSTRACT

The present study deals with a rapid, simple, accurate, and rugged UV spectrophotometric methods have been developed for the estimation of some water insoluble calcium channel blockers using hydrotropic solubilization phenomenon in pure and as well as in dosage forms. The calibration graphs were linear in the 2-20µg/ml concentration range ($r$>0.999) for Nimodipine. The detection was made on 346nm & 357nm in methanol or 1% urea. The method was validated according to ICH guidelines by performing linearity, accuracy, precision, limits of quantitation and selectivity. The results show the method is suitable for its intended use.

**Keywords:** Nimodipine, method validation, simultaneous spectrometric analysis

## INTRODUCTION

Calcium ions play an important part in function of cardiovascular system. Calcium channel blockers primarily exert their activity by inhibiting calcium entry into the cells, there by affecting calcium dependent functions. Calcium channel blockers mainly affect the slow calcium channels of S.A and A.V node, cardiac and vascular smooth muscle cells. As a result, they cause vasodilatation of both the coronary and peripheral arteries. Inhibition of slow calcium channels in nodal and cardiac cells results in decreasing chronotropic and inotropic effect, respectively.

The calcium channel blockers probably enter or cross the cell – membrane in order to gain access to appropriate site of action in or on the channel. There they complex with two

specific groups of membrane site; a low affinity – high capacity group and a high affinity – low capacity group. Nimodipine is chemically O5-(2-methoxyethyl) O3-propan-2-yl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate. It is a yellow crystalline powder. It is soluble in ethyl acetate, sparingly soluble in absolute alcohol Methanol and insoluble in water.

Nimodipine belongs to the class of pharmacological agents known as calcium channel blockers. Nimodipine is indicated for the improvement of neurological outcome by reducing the incidence and severity of ischemic deficits in patients with subarachnoid hemorrhage from ruptured congenital aneurysms who are in good neurological condition post-ictus (e.g., Hunt and Hess Grades I-III). The contractile processes of smooth muscle cells are dependent upon calcium ions, which enter these cells during depolarization as slow ionic transmembrane currents. Nimodipine inhibits calcium ion transfer into these cells and thus inhibits contractions of vascular smooth muscle. In animal experiments, Nimodipine had a greater effect on cerebral arteries than on arteries elsewhere in the body perhaps because it is highly lipophilic, allowing it to cross the blood brain barrier[1, 2]. Nimodipine blocks intracellular influx of calcium that is thought to be a central to ischemic neuronal damage. Nimodipine binds specifically to L-type voltage-gated calcium channels[3].

**Objective**

There is a broad scope for hydrotropic agent in quantitative estimation of poorly water soluble drugs. The present work is an attempt to study and develop spectroscopic methods employing hydrotropic solubilization phenomenon for quantitative estimation and validation of the method according to ICH guidelines[4]. To develop an accurate selective precise and specific methods for estimation of water insoluble calcium channel blockers in bulk and as well as in dosage forms**.**

**Experimental**

**Estimation of Nimodipine in dosage form using hydrotropic solubilization technique**

**Materials & Methods**

**Instrument:** Double beam UV - visible spectrophotometer (Shimadzu UV-1800) with 1 cm matched quartz cells, band pass 1.8 nm, Shimadzu -AX -200 electronic weighing balance and Ultrasonicator, Enertech electronics Pvt. Ltd.

**Drug Sample:** Nimodipine were obtained as gift sample from Sun pharma on top pharmaceuticals limited, Bangalore.

**Chemicals and Reagent:** Methanol A.R. Grade (Loba Chemie, Mumbai). & Urea From SD fine Chemicals Commercially available pharmaceutical dosage form Nimodipine manufactured by USV Limited, B.S.D Marg Govandi, Mumbai-400088 at J-76 MIDC Tarapur, Tal.palghar-401506, Dist Thane.

**Estimation of Nimodipine in pure and in dosage form in methanol**

**Preparation of standard stock solution of Nimodipine in Methanol**

Weighed accurately about 50mg of Nimodepine and transferred into a 50ml standard flask dissolved and made up to the volume using methanol. This solution had a concentration of 1mg per ml of Nimodepine (solution A).

Accurately pipette out 2ml of solution A into a 50ml standard flask and the volume was made up to 50ml using methanol. The resulting solution had a concentration 100mg/ml of Nimodepine (solution B). Accurately pipette out 2ml of solution B separately into 10ml of standard flask and the volume was made up to 10ml using methanol[5]. The resulting solution had a concentration of 20mg/ml of Nimodepine (solution C)[6].

**Study of spectral characteristic of Nimodepine in methanol**

After enabling the initial adjustment and blank correction. Using methanol the solution C was scanned in the entire UV range from 400 to 200 nm. A broad band of absorption spectrum was observed with maximum absorption at 346nm as shown in figure.1.

**Preparation of calibration curve of Nimodepine in methanol**

Accurately pipette out 0.2ml to 1.0ml of solution B into eight 10ml standard flask. Volume was made up to 10ml using methanol. The absorption of each solution was measured at 346nm against reagent blank[7]. The readings were plotted by taking absorbance in Y-axis and concentration X-axis.

**Estimation of Nimodepine from dosage form in methanol**

Twenty tablets containing each of 30mg of Nimodepine were accurately weighed and finally now dried in a glass mortar. A weigh equivalent to 10mg of Nimodepine was accurately transferred to a 10ml of standard flask 4ml of methanol was added and swirled gently for the period of 10min.The clear supernatant solution was then transferred to 10ml standard flask through what mann NO.1 filter paper .The residue was further extracted twice with 2ml each

of methanol and passed the same filter paper and the volume was made up to 10ml with methanol. The resulting solution had a concentration of 1mg/ml (solution A).

Accurately pipette 1ml of the above solution into a 10ml standard flask and made up to volume with methanol. The final solution had a concentration of 100mg/ml of Nimodepine (solution B).

Accurately pipette out 1ml of solution B into 10ml standard flask and the volume was made up using methanol to obtain concentration of 10mg/ml of Nimodepine. The solution was measured at 346 nm and the concentration of Nimodepine was calculated from the calibration graph Table no.1

**Estimation of Nimodipine in pure and in dosage forms by using 1%Urea**

**Preparation of 1% Urea in water**

Weighed accurately about 1 gram of urea and transferred into a 100 ml standard flask dissolved and made up the volume using Distilled water.

**Preparation of standard stock solution of Nimodipine in 1% Urea**

Weighed accurately about 50mg of Nimodipine and transferred into a 50ml standard flask dissolved and made up the volume using 1% Urea. This solution had a concentration of 1mg per ml of Nimodipine (solution A).

Accurately pipette out 5ml of solution A into a 50ml standard flask and the volume was made up to 50ml using 1%Urea. The resulting solution had a concentration 100mg/ml of Nimodipine (solution B)

Accurately pipette out 4ml of solution B separately into 10ml of standard flask and the volume was made up to 10ml using1% Urea .The resulting solution had a concentration of 40mg/ml of Nimodipine (solution C).

**Study of spectral characteristic of Nimodipine in 1%Urea**

After enabling the initial adjustment and blank correction. Using 1% Urea the solution C was scanned in the entire uv range from 400 to 200 nm. A broad band of absorption spectrum was observed with maximum absorption at 357nm as shown in figure.2.

**Preparation of calibration curve of Nimodipine in 1%Urea**

Accurately pipette out .5ml to 4.0ml of solution B into eight 10ml standard flask. Volume was made up to 10ml using 1% Urea .The absorption of each solution was measured at

364nm against reagent blank. The readings were plotted by taking absorbance in Y-axis and concentration X-axis.

**Estimation of Nimodipine from dosage form in 1%Urea**

Twenty tablets containing each of 30mg of Nimodipine were accurately weighed and finally powdered in a glass mortar. A weigh equivalent to 10mg of Nimodipine was accurately transferred to a 10ml of standard flask 4ml of 1% Urea was added and swirled gently for a period of 10min.The clear supernatant solution was then transferred to 10ml standard flask through whatmann NO.1 filter paper .The residue was further extracted twice with 2ml each of 1% Urea and passed the same filter paper and the volume was made up to 10ml with 1% Urea. The resulting solution had a concentration of 1mg/ml (solution A).

Accurately pipette 1ml of the above solution into a 10ml standard flask and made up volume with 1% Urea. The final solution had a concentration of 100mg/ml of Nimodipine (solution B).

Accurately pipette out 1ml of solution B into 10ml standard flask and the volume was made up using methanol to obtain concentration of 10mg/ml of Nimodipine[8]. The solution was measured at 357 nm and the concentration of Nimodipine was calculated from the calibration graph Table No. 1.

**Result and Statistical Evaluation**

The Weight of 20 tablet in methanol & 1% urea is the 1.56gm & 0.9844gm & Each tablet contains (label claim) Nimodepine is 30mg. So that the Average weight of tablet is 0.783 gm & 0.0992 in methanol 7 urea & the Weight of powder taken is 0.0992gm. So that the Average content of Nimodepine /tablet determined by the proposed method is 09.98mg & 0.968 mg. & The Percentage of label claim for methanol and 1% urea is 100.2% & 100.02%.

**Method validation[9, 10]**

**A) Linearity and range**

Calibration curve was prepared for Nimodepine in methanol & 1% Urea at 346nm & 357 nm and entire calibration data at the selected wave length as summarized in table NO.2.These shows that Nimodepine obeyed Beer's law in the concentration range 2 to20mg/ml at 346 nm & 357nm.

**B) Repeatability /precision**

Repeatability expresses the precision under the same operating condition area short interval of time. It is also termed as intraday precision. The precision of the analytical procedure is usually expressed as the standard deviation of a series of measurements [11, 12]. The results obtained from nine observations of the same concentration of sample solution the main volume obtained are tabulated in table No. 3.

**C) Robustness**

The robustness of the method was determined by using Urea from three different suppliers. SD fine chemicals, Mumbai. E.merck. ltd, Mumbai400018. Thomas baker chemicals ltd.mumbai 400002. For the preparation of stock solution of standard drugs and experiment was carried out as mentioned in above & the data obtained is given in Table No.4.

**D) Recovery studies**

Accuracy of the proposed method was determined by performing recovery studies. A fixed amount of drug from dosage form was taken and pure standard drug at three different concentrations within beer's range was added. The total concentration was found by the proposed method. The determination with each concentration was repeated three times and average percent recovery of the added standard was calculated and results are tabulated in table5.

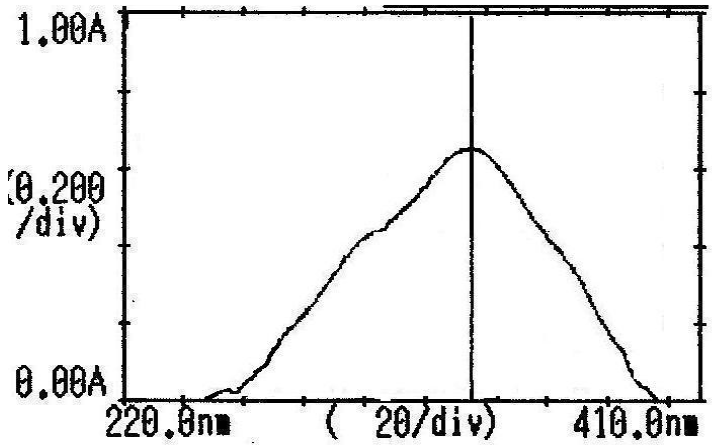


**Fig no 1 Nimodipine in Methanol at 346 nm**

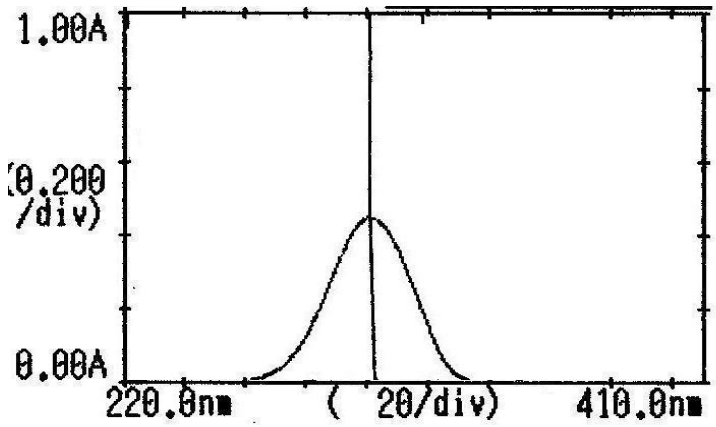


**Fig no 2. Nimodipine in 1% Urea at 357 nm**

**Table No. 1. Results of analysis of Nimodipine in dosage form.**

| Sr. No. | Formulation | Method | Label concentration in µg | Amount of drug found in µg* | %label claim |
|---|---|---|---|---|---|
| **1.** | **Nimodip** | **In Methanol** | 10 | 10.82 | 100.2 |
| **2.** | **Nimodip** | **In 1% Urea** | 10 | 10.042 | 100.02 |

* Average of 5 experiments.

**Table No. 2. Calibration data of Nimodipine at 346 nm and 357 nm.**

| Sr. No. | Parameter | Nimodipine at 346 nm | Nimodipine at 357 nm |
|---|---|---|---|
| 1. | Beer's law limit (µg /ml) | 2 - 20 | 2 - 20 |
| 2. | Regression equation | Y= 0.0455 X +0.0002 | Y= 0.0035 X +0.0162 |
| 3. | Slope | 0.0002 | 0.0162 |
| 4. | Intercept | 0.0455 | 0.0035 |
| 5. | Correlation of coefficient | 0.999 | 0.998 |

**Table No. 3. Precision data of Nimodipine in Methanol and in 1% Urea.**

| Sr. No. | Sample concentration in µg /ml | Concentration obtained by the method | | Mean Value | |
|---|---|---|---|---|---|
| | | In Methanol | In 1% Urea | In Methanol | In 1% Urea |
| 01 | 10 | 9.507 | 9.513 | 7.180 | 9.734 |
| 02 | | 9.450 | 9.685 | | |
| 03 | | 9.058 | 9.456 | | |
| 04 | | 9.214 | 9.325 | | |
| 05 | | 9.451 | 9.789 | | |
| 06 | | 9.825 | 9.014 | | |
| 07 | | 9.432 | 9.350 | | |
| 08 | | 9.364 | 9.114 | | |
| 09 | | 9.652 | 9.451 | | |

- Standard Deviation = 0.00158
- Coefficient of Variance = 0.00667
- Standard Error = 0.000129

**Table No. 4. Robustness with Methanol and 1% Urea .**

| Sr. No. | Methanol | Drug taken in mg | Drug obtained in mg* | % of recovery |
|---|---|---|---|---|
| **1.** | | 10 | 9.035 | 99.31 |
| **2.** | | 10 | 9.952 | 99.47 |
| **3.** | | 10 | 9.892 | 99.44 |
| **Sr. No.** | **1% Urea** | **Drug taken in mg** | **Drug obtained in mg** | **% of recovery** |
| **1.** | | 10 | 10.09 | 100.04 |
| **2.** | | 10 | 10.04 | 100.05 |
| **3.** | | 10 | 9.26 | 99.06 |

**Table No. 5. Recovery Studies of Nimodipine in Methanol and in 1% Urea.**

| Sr. No. | Method | Formulation | Label claim in mg | Concentration of pure drug in mg | Concentration of pure drug found in mg | % Recovery ±SD |
|---|---|---|---|---|---|---|
| 1. | Nimodipine in methanol | | 30 | - | 29.563 | 95.63 |
| | | | 30 | 5 | 35.021 | 99.64 |
| | | | 30 | 10 | 39.652 | 99.82 |
| | | | 30 | 15 | 44.91 | 99.72 |
| 2. | Nimodipine in 1% Urea | | 30 | - | 30.232 | 101.03 |
| | | | 30 | 5 | 35.340 | 102.04 |
| | | | 30 | 10 | 39.831 | 99.90 |
| | | | 30 | 15 | 45.001 | 100.01 |

**ACKNOWLEDGEMENTS**

The authors are thankful to Sun pharma pharmaceuticals limited, Bangalore. for their gift sample of Nimodipine & Management of alwar pharmacy college for providing other necessary requirement for this work.

**REFERENCE**

1. David A William THosmson Lemke. Foye's Principle of Med Chemistary 5th edition Publisher Uppincot Williams and mlking 200 page No 556-558.
2. Indian pharmacopeia Vol II, 5th Edition, 2007, page no. 1442-1445.
3. Anthony C moffat M david Oselton and barin widdap clarke analysia of drug and posion 3rd edition Publisher . The pharmaceutical Pressof Royal pharmaceutical society great Britain UK page 629-630.
4. D. anantha kumar, Simultaneous Determination of Simvastatin and Ezetimibe in Tablets by HPLC, E-Journal of Chemistry 2009, **6(2),** 541- 544
5. Kasutri.V ; Ramkete Madhuri; Simultaneous UV spectrophotometric methods for the estimation of atenolol and nifedipine in solid dosage forms Indian journal of Pharmaceutical Sciences, 2005, vol.67 page 752-754.
6. Bruno L; john SK New spectrophotometric methods for estimation of nifedipine, Indian journal of Pharmaceutical sciences 1988 Mar-Apr;50(2): 109-12.

7. Woolfsan A.D, Maccafferty D. F and Launchbury A. P spectrophotometric determination of Nifedipine , Int. J. Pharm, 1986, 34, 122-127.
8. Maheswari R.K., A novel application of hydrotropic Solubilization in the analysis of bulk samples of ketoprofen and salicylic acid. Asian j.chem 2006,18,393-396
9. M.E. Zorn. R.D.Gibbons and W.C Sonzongi " Evaluation of approximate methods for the calculating the limit of detection and limit of quantitation". Enviorn Sci., Technol. 1999 33.291.
10. P.D Sethi Quantitative analysis of drug in pharmaceutical formulation third edition CBS publishers and distributors, new Delhi, page no. 189.
11. Maheswari R.K., Spectrophotometric determination of Cefixima in tablet by hydrotropic solubilization phenomenon, The Indian pharmacist 2005, vol. 4( No. 36), 63-68.
12. Maheswari R.K., Analysis of furasamide by application of hydrotropic solubilization phenomenon, The Indian pharmacist 2005, vol. 4( No. 34), 55-58.