

## RP-HPLC METHOD FOR ESTIMATION OF AN ANTIPSYCHOTIC DRUG - PIMOZIDE

PRACHI KABRA,<sup>1\*</sup> L.V.G. NARGUND,<sup>1</sup> M. SRINIVASA MURTHY<sup>2</sup>

Nargund College of Pharmacy, Dattatreyanagar, II Main, 100Ft. Ring Road, BSK III Stage, Bangalore-560085, Vignan Institute of Pharmaceutical Sciences, Vigan Hills, Deshmuki Village, Pochampally Mdl, Nalgunda District-508284

Email: prachi.v.kabra@gmail.com

Received: 17 February 2014, Revised and Accepted: 10 March 2014

### ABSTRACT

**Objective:** To develop and validate RP-HPLC method for estimation of Pimozide in bulk and its tablet dosage form.

**Method-** Chromatographic separation was carried out on Grace Smart RP-18 column (250 mm X 4.6 mm inner diameter; 5µm particle size) using a mobile phase consisted of Acetonitrile: 50 mM Disodium hydrogen phosphate buffer (pH 6.2, adjusted by 1 % ortho phosphoric acid) in the ratio of 60: 40 %v/v. The flow rate was maintained at 1 ml/min and UV detection was measured at 280 nm. Propyphenazone was used as an internal standard.

**Results:** The calibration curve of Pimozide was linear in range of 5- 100 µg/ml. The mean % assay of marketed formulation was found to be 101.02 % and % recovery was observed in the range of 99.23- 101.91 %. Relative standard deviation for precision study was found less than 2 %. The LOD and LOQ values were found to be 0.553 µg/ml and 1.678 µg/ml respectively.

**Conclusion:** The developed method is simple rapid and easy to apply, making it suitable for routine analysis of Pimozide in bulk and tablet dosage form.

**Keywords:** Pimozide, RP-HPLC, Validation

### INTRODUCTION

**Pimozide** ( PIMO) is an antipsychotic drug of the diphenyl butyl piperidine class used in Schizophrenia and Tourette syndrome [1]. Chemically it is 1-[1-[4,4-Bis(4-fluorophenyl)butyl]piperidin-4-yl]-1,3-dihydro-2H-benzimidazol-2-one [2]. Chemical Structure of Pimozide is shown in Figure 1. Schizophrenia is a mental health condition that causes disordered ideas, beliefs and experiences. Symptoms of schizophrenia and other similar mental health problems include hearing, seeing, or sensing things that are not real, having mistaken beliefs, and feeling unusually suspicious. Pimozide helps to ease these symptoms [3]. It is also used to reduce uncontrolled movements (motor tics) or outbursts of words/sounds (vocal tics) caused by Tourette syndrome. Pimozide is a medication that works by decreasing the activity of dopamine in the brain [4].

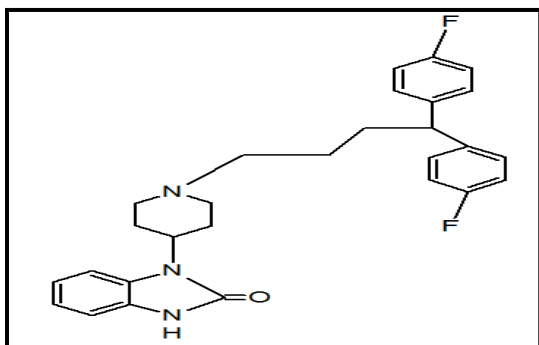


Fig.1: Chemical Structure of Pimozide

Literature survey revealed that few analytical methods are reported for estimation of Pimozide like radioimmunoassay [5], Spectrofluometry [6], HPLC using fluorescence detection for analyzing human plasma [7], Colorimetric method [8], Differential Pulse Voltametric method [9], LC/MS method [10], Stability indicating HPTLC method [11]. It was observed that none of the RP-HPLC method using UV detector and internal standard is reported for the quantification of the Pimozide in tablet dosage form. Hence in present research work attempt has been made for development and

validation of RP-HPLC method for determination of Pimozide using internal standard.

### MATERIAL AND METHODS

#### Chemicals and Reagents

Pimozide working standard powder was kindly provided by Micro Lab Ltd, Bangalore. Pimozide tablets (Mozep-2, Intas Pharmaceutical Ltd) containing 2 mg of Pimozide were purchase from local pharmacy. HPLC grade acetonitrile, methanol and o-phosphoric acid were procured from Merck, India. Analytical grade Disodium hydrogen phosphate (Ranbaxy Fine Chemicals Limited) was used.

#### Instrumentation

The HPLC system used was Shimadzu LC-20AT Pump, SPD-20A UV detector with Rheodyne Injector. The system was controlled through Spinchrome Software. Analytical column used for this method was Grace Smart RP-18 (250 mm X 4.6 mm inner diameter; 5µm particle size). Sartorius Digital Balance, Digisun 7007 pH meter, PCI Sonicator and Vacuum pump were used in the experiment.

#### Chromatographic Conditions

The composition of mobile phase used was Acetonitrile: 50 mM Disodium hydrogen phosphate buffer (pH 6.2, adjusted by 1 % ortho phosphoric acid) in the ratio of 60: 40 %v/v. The mobile phase was vacuum filtered through 0.2 µm Supor 200 membrane filter (Pall life Science) and degassed by ultrasonication for 10 min before use. The mobile phase flow rate was set at 1ml/min. All the solutions were filtered through 0.2 µm Supor 200 membrane filter using syringe. After equilibration with the solvent to obtain a stable baseline, aliquot of samples (20µl) were injected through Rheodyne Injector in the column. The total run time was 10 min. the absorbance of the eluent were monitor at 280 nm with detection sensitivity of 0.001 aufs. Propyphenazone (10 µg/ml) was used as an internal standard.

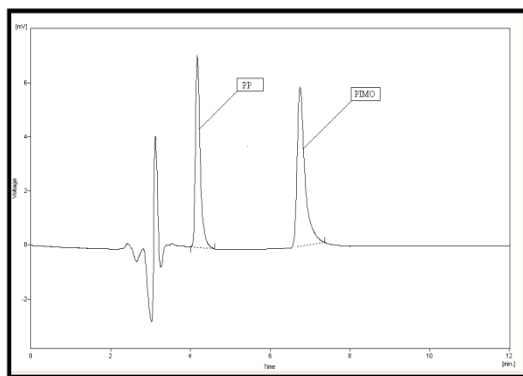
#### Preparation of Standard Stock Solution

The stock solution of Pimozide was prepared by dissolving accurately weighed 25 mg Pimozide in 25 ml of HPLC grade methanol to get concentration of 1000 µg/ml of Pimozide. The stock

solution of Propyphenazone (internal standard) was prepared by dissolving accurately weighed 10 mg of Propyphenazone in 100 ml of HPLC grade methanol to get concentration of 100 µg/ml of Propyphenazone. From this stock solution of Pimozide, serial dilutions for calibration curve in the range of 1- 100 µg/ml (with 10 µg/ml of Propyphenazone as an IS) were prepared using mobile phase.

#### Preparation of Sample Solution

Twenty Tablets of Pimozide (Mozep-2) were weighed accurately and finely powdered. The powder equivalent to 10 mg of Pimozide was transferred to 100 ml volumetric flask. The drug was extracted using ultrasonication for 15min in about 50 ml of HPLC grade methanol. The volume was made upto the mark using same solvent to get concentration of 100 µg/ml of Pimozide. The resulting solution was filtered through whatman filter paper no 41. From the above solution 3 ml of aliquot was transferred to 10 ml volumetric flask along with 1 ml of Propyphenazone solution (100 µg/ml). The volume was made upto the mark with mobile phase to get concentration of 30 µg/ml of Pimozide and 10 µg/ml of Propyphenazone. Chromatogram of Marketed Formulation is shown in Figure 2.



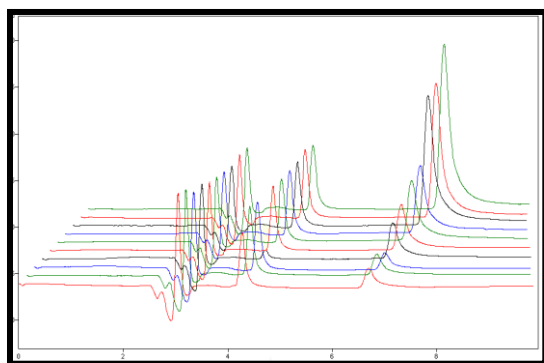
**Fig.2: Chromatogram of Marketed Formulation (Retention Time of Pimozide 6.71 min, Propyphenazone 4.17 min)**

#### Method Validation

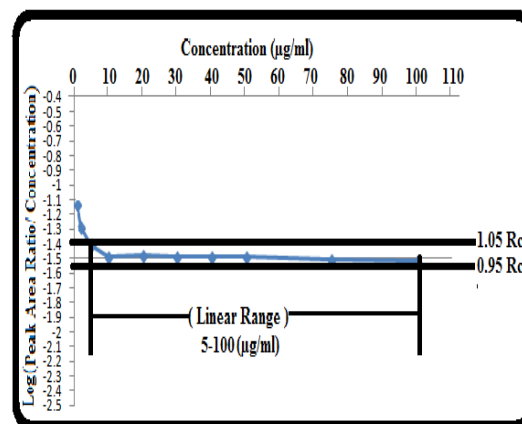
Method validation was carried out under the guidelines of International Conference on Harmonization (ICH).

#### Linearity

Calibration curves were obtained from injecting six sets of ten serial concentrations (1, 2, 5, 10, 20, 30, 40, 50, 75, and 100 µg/ml of Pimozide). Overlain (3D view) Chromatograms of Serial Dilutions of Pimozide is shown in Figure 3. The calibration curves were generated by plotting peak area ratio of Pimozide to Propyphenazone against Pimozide concentration. Linearity of the developed method was evaluated by plotting the log curve as shown in the Figure 4.



**Fig.3: Overlain (3D view) Chromatograms of Serial Dilutions of Pimozide (1-100 µg/ml) with Internal Standard**



**Fig.4: Graph of Linearity Study**

#### Accuracy

In this study, accuracy of the method was determined based on the recovery (percentage) of known amount of standard Pimozide added in the assay samples. This was performed by analysing Pimozide at three different concentration levels 80 %, 100% and 120 % of the assay sample with a constant concentration of 10 µg/ml of internal standard. Samples were prepared in triplicate. The accuracy of the assay was determined by comparing the recovered concentration with the added concentration.

#### Precision

The precision of the method was determined by performing intermediate precision (intraday, interday and variation by different analyst) and repeatability study (n=6) and was expressed as % Relative Standard Deviation (% RSD). Intraday precision was determined by performing nine determinations from triplicate injections of three different concentrations of Pimozide (5, 30, 50 µg/ml) on the same day at different time intervals and on three different days for interday precision. Variation of results by different analyst was checked by performing assay in triplicate by Analyst I and Analyst II and comparing their results by F- test and t- Test.

#### Sensitivity

Sensitivity of the method was determined by means of the detection limits (LOD) and quantification limit (LOQ). Calculations for LOD and LOQ were based on Standard Deviation of y-intercept of the calibration curves ( $\sigma$ ) and average slope of the curve (S), using the equation  $LOD=3.3 \times \sigma/S$  and  $LOQ=10 \times \sigma/S$ .

#### Robustness

Robustness of the method was evaluated by the analysis of Pimozide solution under different experimental conditions such as pH of the mobile phase, flow rate and organic content of the mobile phase. All these parameters were varied by 2% and their effect on the retention time (Rt), tailing factor (T), number of theoretical plates (N) and resolution of the peaks (Rs) were studied.

## RESULTS AND DISCUSSION

#### Mobile Phase Optimisation

Different composition of mobile phases like acetonitrile: water and acetonitrile : 10mM potassium dihydrogen buffer (50:50, 30:70 %v/v) at pH 3 and acetonitrile : 10, 50 mM disodium hydrogen phosphate buffer (50:50, 70:30, 60 :40 %v/v) at pH 6.2 were tried. At pH 3 numbers of theoretical plates and asymmetry of the peak was not satisfactory while, at pH 6.2 it was observed that as acetonitrile ratio decreases the retention time increase and asymmetry of the peak decreases. As mobile phase containing acetonitrile : 50 mM disodium hydrogen phosphate buffer in the ratio of 60 : 40 % v/v at pH 6.2 (adjusted by 1 % ortho phosphoric acid) at flow rate of 1 ml/min gave all satisfactory result so this mobile phase was selected for analysis of Pimozide.

### Assay of Marketed Formulation

Analysis of marketed formulation was carried out using single point analysis method. The mean % assay was found to be 101.02 % and results of the assay are shown in Table 1

**Table 1: Assay Results Marketed Formulation**

Component	Label Claim (mg)	Mean Amount Found (mg) n=6	Mean % Assay $\pm$ SD
PIMO	2.000	2.023	101.02 $\pm$ 0.747

### METHOD VALIDATION

#### Linearity

Linearity of the method was determined using log curve (shown in Figure no. 3) and it was observed in the range of 5-100  $\mu$ g/ml.

#### Accuracy

The results of the accuracy studies are shown in Table 2. % Recovery was found in the range of 99.23- 101.91 % with R.S.D. less than 2%.

**Table 2: Results of Accuracy Study**

% Level of Recovery	Amount of Standard Drug Added ( $\mu$ g/ml)	Mean Amount Recovered ( $\mu$ g/ml) (n=3)	Mean % Recovery (n=3)	% RSD
80%	24	24.17	100.71	1.034
100%	30	30.05	100.18	0.946
120%	36	36.23	100.65	0.997

#### Precision

The % R.S.D. for Intraday, Interday precision and repeatability study was observed less than 2% (Table 3). Variation of results by two different analyst was determined by preparing and measuring the sample solutions of Pimozide (30  $\mu$ g/ml) by Analyst 1 and Analyst 2, separately (n=3). The values obtained were evaluated using F-test and t-test to verify their precision. Calculated values for t-test were found to be 0.790, which is less than the tabulated or standard value (1.533) hence no significant difference was observed between the results of two analysts at probability value of 0.10 (Table 4)

**Table 3: Results of Intraday and Interday Precision Study**

Component	Concentration ( $\mu$ g/ml)	% RSD Intraday (n=3)	% RSD Interday (n=3)
PIMO	5	1.675	1.731
	30	0.250	1.116
	50	0.117	0.191

**Table 4 Results of Variation by Different Analyst**

Mean % Assay $\pm$ SD (n=3)	Result of Analyst I	Result of Analyst II	F-test	t-test	Inference
101.27 $\pm$ 1.024	100.777 $\pm$ 0.397	6.647	0.790	No Significant Difference	

#### Sensitivity

LOD and LOQ of Pimozide were found to be 0.553  $\mu$ g/ml and 1.678  $\mu$ g/ml, respectively. These values are adequate for the detection and quantification of Pimozide.

#### Robustness

For robustness study, the effect of change in the pH (2%) of mobile phase, organic phase ratio (2%) and flow rate (2%) on the retention time, asymmetry factor, theoretical plates and resolution were studied. Standard solutions of Pimozide (30  $\mu$ g/ml) with

Propyphenazone (10  $\mu$ g/ml) was prepared and analyzed at different pH (6.07,6.2,6.32) of the mobile phase, at different organic phase ratio (58.8:41.2, 60:40, 61.2:38.8 %v/v) and at different flow rate (0.98, 1.0, 1.02 ml/min). Percentage RSD of retention time, tailing factor, number theoretical plates and resolution of peak in all three variables was found to be less than 4%.

### System Suitability

System Suitability was performed to confirm that the equipment was adequate for the analysis to be performed. The test was carried out by making six replicate injections of standard solutions of Pimozide (30  $\mu$ g/ml), Propyphenazone (10  $\mu$ g/ml) and analyzing each solute for their peak area, retention time (Rt), theoretical plates (N), resolution (R) and tailing factor (T). The proposed method fulfills these requirements within the accepted limits.

### CONCLUSION

In the present research work to achieve highest precision in quantitative estimation of Pimozide in pharmaceutical dosage form, a reversed phase chromatographic method using internal standard was developed and validated. The method was validated in terms of linearity, accuracy, precision, detection limit, quantification limit, robustness and system suitability. It involves a simple procedure for sample preparation and shorter run time for analytical procedure (10 min). Hence the present RP-HPLC method can be consider a simple, rapid, suitable and easy to apply for routine analysis of Pimozide in pharmaceutical dosage form.

### ACKNOWLEDGEMENTS

The authors shall remain grateful to Micro Labs Ltd, Bangalore for providing Pimozide in the form of gift sample and Nargund College of Pharmacy, Bangalore for providing laboratory and research facilities to complete this project.

### REFERENCES

1. <http://en.wikipedia.org/wiki/Pimozide> (Access Date : 17/10/2013)
2. Indian Pharmacopeia, Government of India, Ministry of Health and Family Welfare, Published by Indian Pharmacopeia Commission Ghaziabad, 2010;1912-4.
3. <http://www.patient.co.uk/medicine/pimozide> (Access Date : 17/10/2013)
4. <http://www.medicinenet.com/pimozide-oral/article.htm> (Access Date : 17/10/2013)
5. Michiels LJM, Heykants JJP, Knaeps AG, Janssen PAJ Radioimmunoassay of the Neuroleptic Drug Pimozide. Life Sci 1975; 16(6):937-44.
6. Baeyens W Determination of Pimozide in Oral Preparation by Spectrofluorimetry. Talanta 1977; 24(9):579-81.
7. Kerbusch T, Desta Z, Soukhova NV, Thacker D, Flockhart DA Sensitive Assay of Pimozide in Human Plasma Using High performance Liquid Chromatography with Fluorescence Detection: Application to Pharmacokinetic Studies. J Chromate B 1997; 694(1):163-8.
8. Kelani K, Bhabwaj LJ, Fattah LA, Ahmed AS Spectrophotometric Determination of Some n-Donating Drugs Using DDQ. Anal Lett 1997; 30(10):1843-60.
9. Ozkan SA, Ozkan Y, Senturk Z Electrooxidation of Pimozide and Its Differential Pulse Voltametric and HPLC-EC Determination. Anal Chem Acta 2002; 453(2): 221-9.
10. Yan M, Li HD, Chen BM, Liu XL, Xu P, Zhu Yg Quantitative Determination of Pimozide in Human Plasma by Liquid Chromatography- Mass Spectrometry and Its Application to a Bioequivalence Study. J Pharm Biomed Anal 2010; 61(6):1161-4.
11. Devi ASA, Ravi TA. Stability Indicating HPTLC Method of Pimozide in Bulk and Pharmaceutical Dosage Form. Der Pharm Lett 2013; 5(3): 182-7.