Research Article www.ijcps.com

International Journal of Chemical and Pharmaceutical Sciences
2014, June., Vol. 5 (2)

ISSN: 0976-9390
IJCPS

Spectrophotometric determination of pimozide in pure and pharmaceutical forms using triphenyl methane dyes

[1]Sayanna, [1]Veeraiah T[*] and [2]Venkata Ramana Reddy Ch.

[1]Department of Chemistry, SAP College, Vikarabad, Ranga Reddy Dist, Telangana, India.

[2]Department of Chemistry, JNTUH College of Engineering, Kukatpally, Hyderabad, Telangana, India.

[*] **Corresponding Author:** E-Mail: tadooru_veeraiah@rediffmail.com

Abstract

Three simple and sensitive extractive spectrophotometric methods for the determination of Pimozide in pure form and in pharmaceutical formulations have been described and presented in this paper. The developed methods involve formation of coloured chloroform extractable ion-pair complexes of the drug with triphenyl methane dyes *viz.,* bromophenol blue (BPB), bromothymol blue (BTB) and bromocresol purple (BCP) in acidic medium. The extracted complexes formed with BPB, BTB and BCP showed absorbance maxima at 410, 415 and 419nm respectively**.** The stoichiometry of the ion- pair complex is found to be 1:1 in each case. Beer's law is obeyed in the concentration ranges 2.0-25, 3.0-30 and 4.0-40 µg/ml with BPB, BTB and BCP respectively. The effects of concentration of dye, pH, and interference of excipients have been studied for optimization. The limits of detection and quantification have been determined for all the three methods. These methods have been validated as per the guidelines of ICH. The methods have been applied to the determination of drug in commercial tablets and results of analysis were validated statistically through recovery studies.

Keywords: Spectrophotometry, Pimozide, Bromophenol blue, Bromothymol blue, Bromocresol purple, Ion-pair complex, Validation.

1. INTRODUCTION

Chemically, Pimozide is 1-[1-[4,4-Bis(4-fluorophenyl)butyl]piperidin-4-yl]-1,3-dihydro-2*H*-benzimidazol-2-one[1]. Chemical Structure of Pimozide is shown in figure 1. It is an antipsychotic drug of the diphenyl butyl piperidine class used in Schizophrenia and Tourette syndrome[2]. 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]. At the low-dose level Pimozide is a highly selective blocker of central dopamine receptors and increases the turnover of dopamine in the central nervous system, at higher doses, increased turnover of noradrenaline has also been observed. In addition, Pimozide has a reported calcium channel-blocking ability. This property contributes to Pimozide's tendency to prolong the ECG QT interval[5]. Neurologic side effects of Pimozide appear to be less severe than those observed from other drugs[6]. Pimozide has been used in the treatment of delusional disorder and paranoid personality disorder[7]. It has also been used for delusions of parasitosis[8] and as a Listeria monocytogenes inhibitor[9].

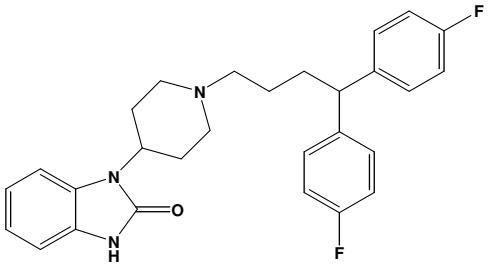


Figure - 1: Chemical structure of pimozide.

Several analytical methods for the estimation of Pimozide like radioimmunoassay[10], Spectroflurometry[11], HPLC using fluorescence detection for analyzing human plasma[12], Differential Pulse Voltametric method[13], LC/MS method[14], Stability indicating HPTLC method[15] are available in the literature. Recently, Kabra et al. reported RP-HPLC method for the estimation of Pimozide[16]. The chemical features of the drug molecule offers a lot of scope for the development of new methods for its determination with better sensitivity, specificity, precision and accuracy. The reported chromatographic techniques require expensive experimental set-up and are not affordable in every laboratories for routine analysis. The literature survey revealed that, although, spectrophotometric methods for the determination of Pimozide using $[Cr(NCS)_6]^{3-}$[17] and DDQ[18] are available, a little attention was paid to the development of spectrophotometric methods for its determination using dyes. Spectrophotometry is considered as the most convenient analytical technique because of its inherent simplicity, low cost, and wide availability in most quality control laboratories. So the present study reports on newly developed and validated spectrophotometric methods for the estimation of Pimozide in bulk and pharmaceutical formulations using triphenyl methane dyes *viz.,* bromophenol blue (BPB), bromothymol blue (BTB) and bromocresol purple (BCP). The developed methods involve formation of coloured chloroform extractable ion-pair complexes of the drug with dyes in acidic medium. The proposed methods have the advantages of speed and simplicity besides being accurate and precise, and can be adopted by the pharmaceutical laboratories for industrial quantitative analysis of Pimozide.

2. EXPERIMENTAL

2.1. Material and Methods

Pimozide was procured from Srini Pharmaceuticals Limited, Hyderabad as a gift sample. The dyestuffs *viz.,* BPB, BTB and BCP (AR grade) supplied by SD Fine Chemicals Ltd. Mumbai, were used without any further purification. The dyestuffs were used as 0.025% solutions in doubly distilled water. Sodium acetate-hydrochloric acid buffers (Britton, 1942) of *p*H 2.5, 3.0 and 3.5 were prepared by mixing 50ml of 1.0*M* sodium acetate solution with calculated volume of 1.0 *M* HCl solution and diluted to 250 ml with doubly distilled water. The *p*H of each solution was adjusted to an appropriate value with the aid of a *p*H meter. Chloroform (HPLC grade) supplied by SD Fine Chemicals Ltd. Mumbai was used throughout the work.

The spectra (Figures 2 - 4) of ion-pair complexes have been recorded on Elico double beam SL 210 spectrophotometer using quartz cells of 10 mm path length. An Elico model Li-120 *p*H meter was used for *p*H measurement.

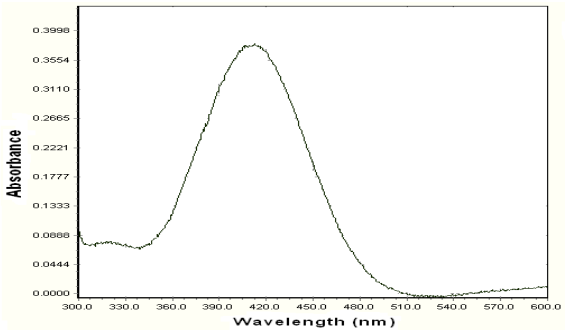


Figure - 2: Absorption spectrum of pimozide-bromophenol blue (BPB) complex extracted into 10 ml chloroform ([drug] = 25 µg ml^{-1} + 5 ml of 0.025% BPB + 5 ml of pH 2.5 buffer).

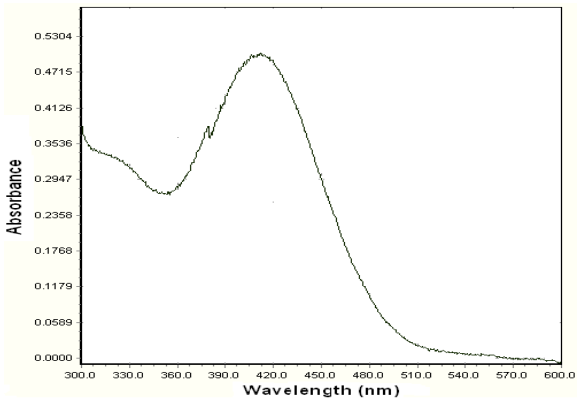


Figure - 3: Absorption spectrum of pimozide-bromothymol blue(BTB) complex extracted into 10 ml chloroform ([drug] = 25 µg ml^{-1} + 5 ml of 0.025% BTB + 5 ml of pH 2.8 buffer).

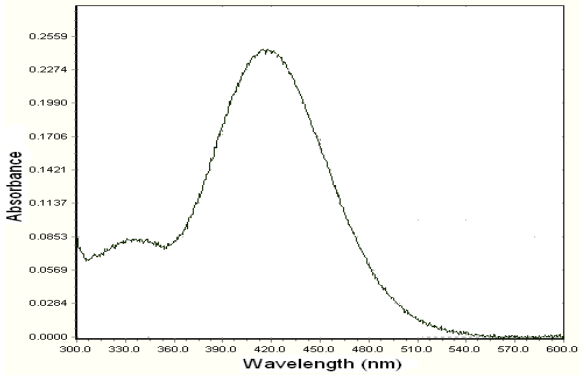


Figure - 4: Absorption spectrum of pimozide-bromocresol purple (BCP) complex extracted into 10 ml chloroform ([drug] = 25 µg ml^{-1} + 5 ml of 0.025% BCP + 5 ml of pH 3.5 buffer).

Table -1: Optical characteristics and statistical analysis for the regression equation of the proposed methods

Parameters	Extraction methods with[b]		
	BPB	BTB	BCP
λ_{max} (nm)	410	415	419
Beer's law limit (µg ml^{-1})	2.0-25	3.0-30	4.0-40
Molar absorptivity (L mol^{-1} cm^{-1})	17522	19323	21930
Formation constant, K, M^{-1}	$1.39x10^6$	$1.61x10^6$	$1.71x10^6$
Sandell sensitivity (µg cm^{-2})	0.0153	0.0132	0.0122
Slope (specific absorptivity), b	0.0502	0.0701	0.0782
Intercept (a)	0.0052	0.0231	0.0222
Correlation coefficient (r)	0.998	0.999	0.998
Standard deviation of intercepts (% n=6)	0.0057	0.0069	0.0112
Limit of detection, µgml^{-1}	0.2355	0.3561	0.4375
Limit of quantification, µgml^{-1}	0.7532	1.0925	1.2413
Regression equation[a]	Y=0.0502C± 0.0052	Y=0.0701C± 0.0231	Y=0.0782C± 0.0222

[a]With respect to *Y=bc+a*, where C is the concentration (µg ml^{-1}) and *Y* is absorbance; [b]Six replicate samples.

Table - 2: Application of proposed methods for the analysis of pimozide in pure form

Taken (µg ml^{-1})	Proposed method						Reference method
	Found (µg ml^{-1})			Recovery %			Recovery %
	BPB	BTB	BCP	BPB	BTB	BCP	
5	5.09	4.97	5.12	101.8	99.4	102.4	101.2
10	9.98	10.11	10.15	99.8	101.1	101.5	99.8
15	14.96	14.95	15.12	99.73	99.73	100.8	101.38
20	20.15	19.85	19.23	100.75	99.25	99.25	101.12
25	25.07	25.15	24.98	100.28	100.28	99.92	101.2
							99.86
							101.85
							99.98
RSD (%)				0.8445	0.7540	1.2377	0.7880
Mean±SD				100.47 ±0.8485	99.95 ±0.7537	100.77 ±1.2473	100.79 ±0.7943
t-test				0.1084	0.0881	0.6691	
F-test				0.8763	1.1107	0.4057	

2.2. Calibration curve

Different aliquots of drug solution were transferred into 125 ml separating funnel. To this 5 ml of buffer (*p*H 2.5, 3.0 and 3.5), 5 ml of dye were added and total volume was made up to 20 ml with water. 10 ml of chloroform was added and the contents were shaken for 5 min. The two layers were allowed to separate for 5 min. The organic layer was separated and absorbance of yellow colored solution which is stable atleast for 3 hrs is measured at 419 nm against blank similarly prepared. The same procedure of analysis is followed either for assay of pure drug or for dosage form. The calibration graphs (Figure 5) are linear over the concentration ranges and are within the permissible range. The optical characteristics and statistical data for the regression equation of the proposed methods are presented in table 1.

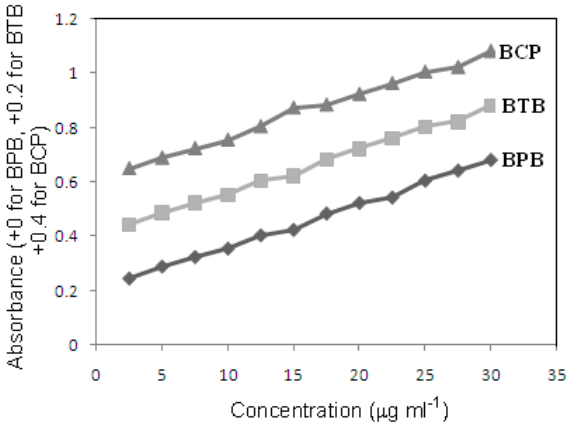


Figure - 5: Calibration graphs for Drug-BPB, BTB & BCP Ion-pair complexes.

2.3. Procedure for the assay of pure drug

Five different solutions of pure drug in the range of calibration curve were selected and the recovery experiments were performed. The recoveries and their relative standard deviations are tabulated in table 2.

2.4. Procedure for the assay of dosage forms

Ten tablets of Orap 4mg each are powdered and dissolved in doubly distilled water and stirred thoroughly, filtered through a Whatman No. 42 filter paper. This solution was transferred into 100 ml standard volumetric flask and diluted with doubly distilled water as required. Different solutions of drug in the range of calibration curve were chosen and the assay was estimated using the calibration curve. The results of the recovery experiments are tabulated in table 3.

Table - 3: Application of proposed methods for the analysis of Pimozide in pharmaceutical form

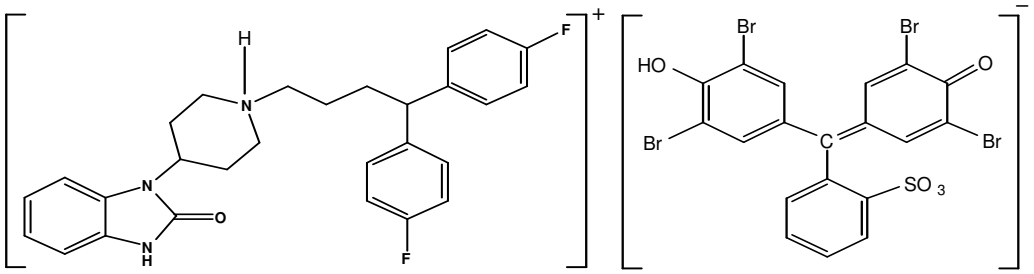
	Proposed method						Reference method
Taken (µg ml^{-1}) Orap 4mg	Found (µg ml^{-1})			Recovery (%)			Recovery (%)
	BPB	BTB	BCP	BPB	BTB	BCP	
4	3.98	4.08	3.97	99.5	102	99.26	98.96
8	8.05	7.98	8.12	100.6	99.75	101.5	101.15
12	11.98	12.05	12.05	99.83	100.4	100.4	101.24
16	16.05	16.15	15.98	100.3	100.9	99.87	101.12
20	20.02	19.98	19.85	100.1	99.9	99.25	101.25
							100.35
							99.98
							101.48
							99.75
RSD (%)				0.4206	0.9031	0.9377	0.8635
Mean±SD				100.66 ±0.4234	100.59 ±0.9085	100.05 ±0.9382	100.58 ±0.8686
t-test				1.2414	0.0835	0.1355	
F-test				0.2376	0.9195	0.8570	

3. RESULTS AND DISCUSSION

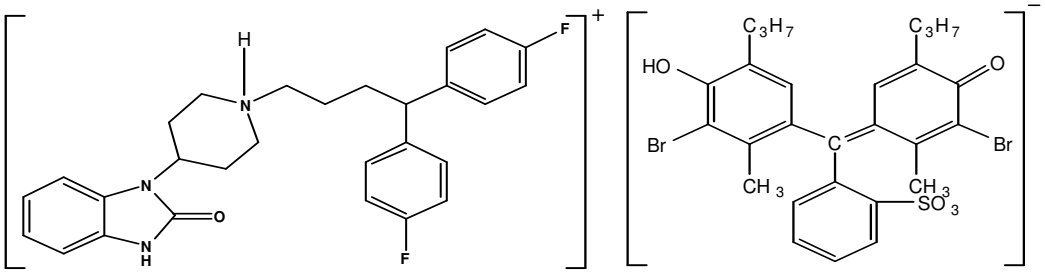
3.1. Formation of Ion-pair complexes

Pimozide forms ion-pair complexes in acidic buffer with dyestuffs such as bromophenol blue (BPB), bromothymol blue (BTB) and bromocresol purple (BCP) and these complexes are quantitatively extracted into chloroform. Ion-pair complexes of drug with BPB, BTB and BCP absorbed maximally at 410, 415 and 419nm respectively (Figures 2 - 4). The reagent blank under similar conditions showed no absorption.

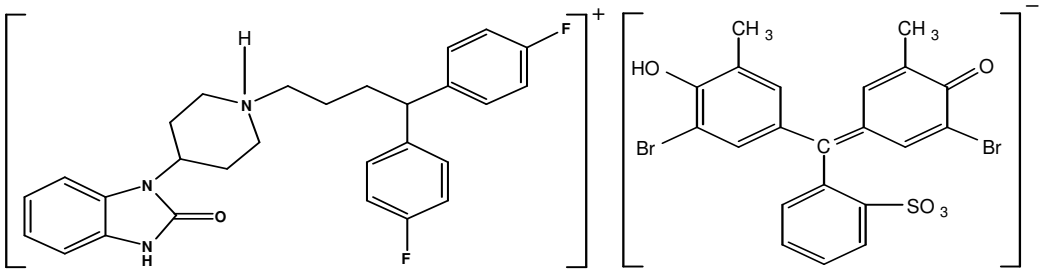
Pimozide contains piperidine nitrogen which is protonated in acid medium, while sulphonic acid group present in BPB, BTB and BCP undergoes dissociation in the *p*H range 1-5. The colour of the ion-pair complex is attributed to opening of lactoid ring and subsequent formation of quinoid group in dye moiety. It is appropriate to mention that the two tautomers are present in equilibrium but due to strong acidic nature of the sulphonic acid group, the quinoid body must predominate. Finally the protonated Pimozide forms ion-pairs with the dyestuffs which are quantitatively extracted into chloroform. Figure 6 shows the possible structures of ion-pair complexes.



Pimozide-Bromophenol blue ion-pair complex



Pimozide-Bromothymol blue ion-pair complex



Pimozide-Bromocresol purple ion-pair complex

Figure - 6: Pimozide-Dye ion-pair complexes.

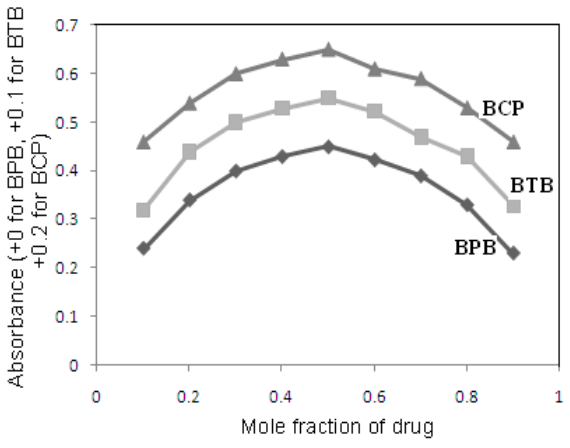


Figure - 7: Continuous-variations study of drug-dye systems ([Drug] = [Dye] = 8x10^{-5}M).

3.2. Stoichiometry

In order to establish molar ratio between Pimozide and dyestuffs used, the Job's method[19] of continuous variation has been applied. In this method, solutions of drug and dyestuff with identical molar concentrations (8 x 10^{-5}*M*) were mixed in varying volume ratios in such a way that the total volume of each mixture was the same. The absorbance of each solution was measured and plotted against the mole fraction of the drug, [drug]/ [drug] + [dyestuff] (Figure 7). This measurement showed that 1:1 complex was formed with each dyestuff. The formation constants[20], [21] were also estimated and found to be 1.39x 10^6, 1.61x 10^6 and 1.71x 10^6 K M^{-1} for complexes with BPB, BTB and BCP respectively.

3.3. Optimization of the factors affecting the absorbance

The influence of *p*H on the ion-pair formation of Pimozide with various dyestuffs has been studied using sodium acetate-hydrochloric acid buffer. The results are shown in figure 8. It is evident that absorbance of complexes with BPB, BTB and BCP was found to be constant within the *p*H ranges 2.0-3.0, 2.5-3.5 and 3.0-4.0 respectively.

Thus, all the absorbance measurements were made at *p*H 2.5, 2.8 and 3.5 with BPB, BTB and BCP respectively.

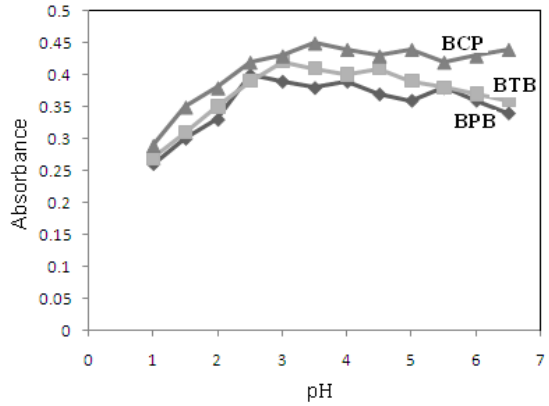


Figure - 8: Effect of pH ([Drug] = [8µg ml^{-1}, [Dye] = 5ml of 0.025%).

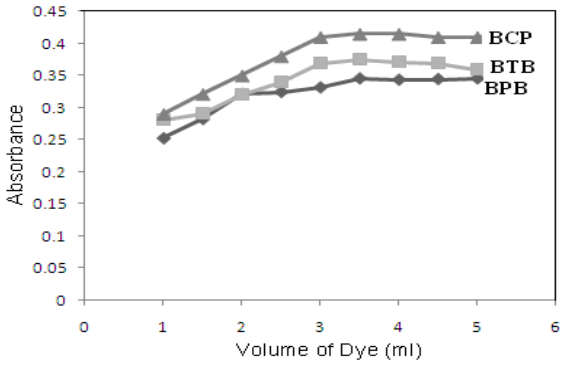


Figure - 9: Influence of the volume of 0.025% Dye ([Drug] = [8µg ml^{-1}]).

The effect of dyestuff concentrations was also studied by adding different volumes of dyestuff to a constant amount of Pimozide (8 µg ml^{-1}). It is apparent from figure 9 that the maximum absorbance, in each case, was found with 3.5 ml of dyestuff, beyond which absorbance was constant. Thus, 5 ml of each dyestuff was used for ion-pair formation throughout the experiment.

Table - 4: Interference study

Excipients	Tolerance limit (µg ml^{-1})
Microcrystalline cellulose	85
Starch	145
Lactose	115
Povidone	48
Fumed silica	75
Titanium dioxide	45
Magnesium stearate	42

A systematic study of the effect of foreign species present along with Pimozide on the determination of Pimozide at 8 µg ml^{-1} levels was undertaken. This study was carried out by following the proposed procedures for a 10 ml sample system, by adding a known amount of foreign species to a pimozide solution of 8 µg ml^{-1}. Table 4 summarizes the results obtained. However, the drug content from the powdered capsules was extracted into chloroform, which completely removes any interference by the common excipients found in formulations.

3.4. Validation of the proposed method

All the three proposed methods have been validated in terms of guideline proposed by International Conference on Harmonization[22] *viz*. selectivity, specificity, accuracy, precision, limits of calibration curve, LOD, LOQ, robustness, ruggedness and regression equation. The student t-test and variance F-test have been performed in comparison with a reference method. Table 1 summarizes the values for Beer's law limits, molar absorptivity, regression equation, correlation coefficients, relative standard deviation and recoveries. To test the reproducibility of the proposed methods, six replicate determinations of 10µg ml^{-1} of Pimozide were made. The coefficient of variation was found to be less than 1.2% for all the procedures.

The proposed methods have been successfully applied to the determination of Pimozide in pharmaceutical preparations. The performance order of the proposed methods is BPB>BTB>BCP. The results obtained and shown in Table 2 and Table 3 were compared to those obtained by a reference method[22] by means of *t*-test at 95% confidence level. In all cases, the average results obtained by proposed methods and reference method were statistically identical, as the difference between the average values had no significance at 95% confidence level.

4. CONCLUSION

Pimozide forms ion-pair complexes with acidic triphenylmethane dyes *viz.,* bromophenol blue, bromothymol blue and bromocresol purple in 1:1 proportion. These complexes are extractable into chloroform and their maximum absorbances offer a basis for assay of the drug. The developed methods are simple, sensitive, reproducible and can be used for routine analysis of Pimozide in pure and pharmaceutical dosage forms.

Acknowledgement

The authors are grateful to Prof. G. Venkateshwarlu, Department of Chemistry, Osmania University, Hyderabad for helpful discussion and to Sri M. Ravindra Reddy, Chairman, Managing Committee SAP College, Vikarabad for providing facilities. The authors are

thankful to the UGC for financial assistance under Major Research Project.

5. REFERENCES

1. Indian Pharmacopeia. Government of India, Ministry of Health and Family Welfare, Published by **Indian Pharmacopeia Commission, Ghaziabad,** 2010; 1912-19124.
2. Muller N, Riedel M, Zawta P, Gunther W and Straube A. Comorbidity of Torette's syndrome and schizophrenia – biological and physiological parallels. **Progress in Neuro-psychopharmacology and Biological Psychiatry**. 2002; 26(7,8): 1245-1252.
3. Kerbeshian J, Zi Peng Chun and Burd L. Tourette syndrome and comorbid early-onset schizophrenia. **Journal of Phsych. Res**. 2009;67(6):515-523.
4. Pecina S, Berridge KC and Parker. Pimozide does not shift palatability: Separation of Anhedonia from sensorimotor suppression by taste reactivity. **Pharmacology Biochem. & Behav.** 1997; 58(3): 801-811.
5. Gould RJ, Murphy KM, Reynolds IJ and Snyder SH. Antischizophrenic drugs of the diphenylbutylpiperidine type act as calicium channel antagonists, **Proc. Natl. Acad. Sci. USA.** 1983; 80(16): 122-5125.
6. Sallee FR, Nesbitt L and Jackson C. Relative efficacy of Haloperidol and Pimozide in children and adolescents with Tourette's disorder. **Am. J. Psychiatry**. 1997; 154: 1057-1062.
7. Munro A. **Delusional disorder.** Cambridge: Cambridge University Press. 1999.
8. Van Vloten WA. Pimozide: Use in dermatology. **Dermatol. Online J**. 2003; **9**(2).
9. Lieberman LA and Higgins DE. (February 2009). A small –molecule screen identifies the antipsychotic drug pimozide as an inhibitor of listeria monocytogenis infection. **Antimicrob. Agents Chemother.** 2009; **53**(2): 756–64.
10. Michiels LJM, Heykants JJP, Knaeps A and Janssen PAJ. Radioimmunoassay of the Neuroleptic Drug Pimozide. **Life Sci.** 1975; 16(6):937-44.
11. Baeyens W. Determination of Pimozide in Oral Preparation by Spectrofluorimetry. **Talanta**. 1977; 24(9): 579-81.
12. Kerbusch T, Desta Z, Soukhova NV, Thacker D and 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): 163-168.
13. Ozkan SA, Ozkan Y and Senturk Z. Electrooxidation of Pimozide and Its Differential Pulse Voltametric and HPLC-EC Determination. **Anal Chem Acta**. 2002; 453(2): 221- 229.
14. Yan M, Li HD, Chen BM, Liu XL, Xu P and Zhu Yg. Quantitative Determination of Pimozide in Human Plasma by Liquid Chromatography-Mass Spectrometery and Its Application to a Bioequivalence Study. **J. Pharm. Biomed. Anal**. 2010; 61(6): 1161-4.
15. Devi ASA abd Ravi TA. Stability Indicating HPTLC Method of Pimozide in Bulk and Pharmaceutical Dosage Form. **Der. Pharm. Let**. 2013; 5(3): 182-187.
16. Kabra P, Nargund LVG and Srinivas Murthy M. RP-HPLC method for estimation of an antipsychotic drug-Pimozide. **Asian J. Pharmaceutical and Clinical Res**. 2014; 7(2): 49-51.
17. Kurzawa M, Kowalczyk-MA and Edward S. Conductometric and spectrophotometric determination of haloperidol, droperidol and pimozide. **Chem. Anal. (Warsaw).** 2004; 49: 91-99.
18. Kelani K, Bhbawy LJ, Fattah LA and Ahmed AS. Spectrophotometric Determination of Some n-Donating Drugs Using DDQ. **Anal. Let**. 1997; 30(10): 1843-1860.
19. Vosburgh, WC and Coopper, GR. The identification of complex ions in solution by spectrophotometric measurements. **J. Am. Chem. Soc**, 1941; 63: 437-442.
20. Likussar W and Boltz DF. Theory of continuous variations plots and a new method for spectrophotometric determination of extraction and formation constants. **Anal. Chem**. 1971; 43**:**1265-1272.
21. Momoki K, Sekino J , Sato H and Yamaguchi N. Theory of curved molar ratio, plots and new linear plotting method. **Anal. Chem,** 1969; 41: 1286-1299.
22. **ICH** (International Conference on Harmonization) of Technical Requirement for the Registration of Pharmaceuticals for Human use, Validation of analytical procedures: definitions and Terminology Genera. 1996.