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Determination of tramadol hydrochloride and its preparations by acid-base titration in non-aqueous medium

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Abstract: Tramadol hydrochloride (TMH) is a centrally-acting synthetic analgesic with μ-opioid receptor agonist activity, and is a widely prescribed analgesic used in the treatment of moderate to severe pain and an alternative to opiates. Two simple, rapid, reliable, precise and accurate and cost-effective non-aqueous titrimetric procedures have been developed for the determination of TMH in bulk drug and its pharmaceutical formulations. The methods are based on the titration of the drug in glacial acetic acid in the presence of mercuric acetate with acetous perchloric acid to the visual end point, using crystal violet as indicator and to the potentiometric end point. The methods were applicable over the range of 1-20 mg TMH. The procedures were also applied for the determination of TMH in its dosage forms and the results were found to be in a good agreement with those obtained by the reference method. The precision results, expressed by intra-day and inter-day relative standard deviation values, were satisfactory (RSD < 2.0%). The accuracy was also satisfactory (RE ≤ 1.76%). Excipients used as additives in pharmaceutical formulations did not interfere in the proposed procedures as shown by the recovery study *via* standard addition technique with percentage recoveries in the range 98.36-107.3 % with a standard deviation of ≤ 1.34 %.

Key words: tramadol hydrochloride, determination, titrimetry, perchloric acid pharmaceuticals

INTRODUCTION

Tramadol hydrochloride (TMH), chemically known as (1R, 2R)-rel-2-[(Dimethylamino)methyl]-1-(3-methoxyphenyl)cyclohexanol, is a synthetic analogue of codeine. It is a centrally-acting analgesic agent [1]. It has been in use since 1977 for relief of severe physical pain and has been the most widely sold opioid analgesic drug in world [2], officially listed in the British Pharmacopoeia [3].

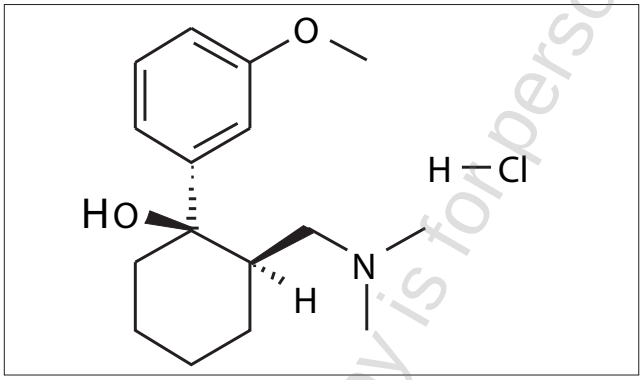

Figure 1 Structure of TMH.

Because of its wide use, several techniques have been reported for its assay in pharmaceuticals and include high performance liquid chromatography [4-7], thin-layer chromatography-densitometry [8], capillary isotechophoresis [9], ion-selective electrode based potentiometry [10-16], adsorptive stripping voltammetry [17], square wave voltammetry [18], uv-spectrophotometry [4, 19, 20], flow injection chemiluminescence spectrophometry [21] and visible spectrophotometry [22-24]. Most of the methods [4-18] are deficient on simplicity, cost-effectiveness and easy accessibility. The reported visible spectrophotometric methods, besides being less sensitive, require heating at 60°C for 40 min [22], involve careful adjustment of experimental conditions being kinetic methods, [23] or require close pH control and liquid-liquid extraction with organic solvent [24].

Titrimetry is still widely used in analytical chemistry for its superior speed and simplicity with little sacrifice in accuracy and precision. TMH is present in relatively large amounts (50 and 100 mg per tablet) in pharmaceutical formulations. Moreover, the instrumental method is generally not as accurate and precise as the titrimetry in macroanalysis. In spite of these advantageous features, titrimetry has not been applied for the assay of TMH in pharmaceuticals.

In the present paper, two simple, rapid, accurate and precise titrimetric methods are presented for the assay of TMH in pure drug and in dosage forms. The methods involve the titration of the acetous drug solution with acetous $HClO_4$ in the presence of mercuric acetate, and the end point being determined either visually using crystal violet as the indicator or potentiometrically using the glass modified saturated calomel electrode (SCE) system. The method validation was studied and the validated methods applied to determine the drug in different TMH preparations with good recoveries and high precision.

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EXPERIMENTAL

Apparatus. An Elico 120 digital pH meter provided with a combined glass-SCE electrode system was used for potentiometric titration. The KCl of the salt bridge was replaced with saturated solution of KCl in glacial acetic acid.

Reagents and Standards. All chemicals used were of analytical reagent grade. All solutions are made in glacial acetic acid (S. D. Fine Chem, Mumbai, India) unless otherwise stated.

Perchloric Acid (0.01 M). A stock solution of (~0.1 M) perchloric acid (S. D. Fine Chem, Mumbai, India) was diluted appropriately with glacial acetic acid to obtain a working solution of 0.01 M perchloric acid, and standardized with pure potassium hydrogen phthalate and crystal violet as indicator [25].

Crystal violet indicator (0.1%). Prepared by dissolving 50 mg of dye (S. D. Fine Chem, Mumbai, India) in 50 mL of glacial acetic acid.

Mercuric acetate solution (3%). Three grams of the pure $Hg(OAc)_2$ (Merck,Mumbai,India) was dissolved in 100 mL of glacial acetic acid, filtered and used.

TMH Standard drug solution (2mgml^{-1}). Stock standard solution containing 2 mg mL^{-1} of the drug was prepared by dissolving the required amount of TMH (Jubiliant Organosys Pvt.Ltd.,Mysore,India) in glacial acetic acid.

PROCEDURES

Visual Titration (Method A). An aliquot of the drug solution containing 1.0-20.0 mg of TMH was measured accurately and transferred into a clean and dry 100 mL titration flask and the total volume was brought to 10 mL with glacial acetic acid. Then, 3 mL of 3% $Hg(OAc)_2$ was added, the content was mixed, and after 2 min, two drops of crystal violet indicator were added and titrated with standard 0.01 M perchloric acid to a blue end point. The amount of the drug in the measured aliquot was calculated based on the formula:

$$Amount(mg) = \frac{VM_wR}{n}$$

where V = volume of perchloric acid required, mL; M_w = relative molecular mass of the drug; and R = molarity of the perchloric acid and n = number of moles of perchloric acid reacting with each mole of TMH.

Potentiometric Titration (Method B). An aliquot of the standard drug solution equivalent to 1.0-20.0 mg of TMH was measured accurately and transferred into a clean and dry 100 mL beaker and the solution diluted to 25 mL by adding glacial acetic acid followed by the addition of 3 mL of 3% $Hg(OAc)_2$. The combined glass-SCE (modified) system was dipped in the solution. The content was stirred magnetically and the titrant (0.01 M $HClO_4$) was added from a microburette. Near the equivalence point, the titrant was added in 0.05 mL increments. After each addition of titrant, the solution was stirred magnetically for 30 s and the steady potential noted. The addition of titrant was continued until there was no significant change in potential on further addition of titrant. The equivalence point was determined by applying the graphical method. The amount of the drug in the measured aliquot was calculated as described under visual titration.

Procedure for Tablets. Four brands of tablets, namely, Tramazac-TC 100 (Zydus Alidac Pvt Ltd., Bangalore, India), Contramol-DT (Piramal Healthcare), Cemadol 50 CR (Life Medicare & Biotech Pvt. Ltd., Haridwar, India), Trambax 50 Oro-Dispersible (Ethypharm LL Pvt. Ltd., Ambarnath (W), Thane, India) were purchased from the local commercial sources and used in the investigation.

Twenty tablets were weighed and ground into a fine powder. An amount of powder equivalent to 100 mg of TMH was weighed accurately into a 100 mL calibrated flask, 70 mL of glacial acetic acid was added and shaken for about 20 min. Then the volume was made upto the mark with glacial acetic acid, mixed well and filtered using Whatmann No 42 filter paper. The first 10 mL portion of the filtrate was discarded. A 10ml aliquot of the filtrate was next subjected to analysis by titrimetry as described earlier.

RESULTS AND DISCUSSION

The present methods are based on the neutralization reaction involving the basic property of TMH and employ two equivalence point detection procedures.

Acetic acid displays acidic properties in dissociating to produce protons [26]:

$$CH_3COOH \longleftrightarrow CH_3COO^- + H^+$$

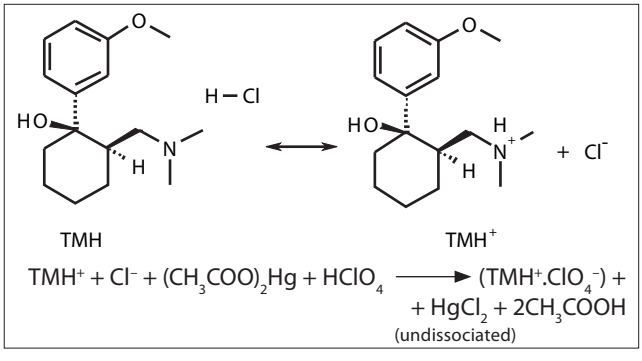
But in the presence of perchloric acid, a far stronger acid, it will accept a proton:

$$CH_3COOH + HClO_4 \longleftrightarrow CH_3COOH_2^+ + ClO_4^-$$

$$2CH_3COOH_2^+ + 2CH_3COO^- \longleftrightarrow 4CH_3COOH$$

The $CH_3COOH_2^+$ can very readily give up its proton to react with a base, therefore the basic properties of a base are enhanced and hence, titration between weak base and perchloric acid can often be accurately carried out using acetic acid as solvent.

Since, the TMH is a hydrochloride, which is a very weakly basic, it cannot react quantitatively with acetous perchloric acid. In order to overcome this problem, mercuric acetate was added (which remains undissociated in acetic acid solution) to TMH solution, thereby causing the replacement of the chloride ion by an equivalent amount of acetate ion which serves as a strong base in acetic acid as, shown in the scheme given below:



Scheme Possible reaction scheme for the neutralization.

The enhanced basicity of TMH in acetic acid medium is due to the non-levelling effect of acetic acid and the determination of TMH is very easier. The procedures involve the titration of TMH with perchloric acid with visual and potentiometric end point detection. Crystal violet gave satisfactory end point for the concentrations of analyte and titrant employed. A steep rise in the potential was observed at the equivalence point with potentiometric end point detection (Fig. 1). With both methods of equivalence point detection, a reaction stoichiometry of 1:1 (drug:titrant) was obtained which served as the basis for calculation. Using 0.01 M perchloric acid, 1.0-20.0 mg of TMH was conveniently determined. The relationship between the drug amount and the titration end point was examined. The linearity between two parameters is apparent from the correlation coefficients of 0.09976 and 0.9984 obtained by the method of least squares for visual and potentiometric methods, respectively. From this it is implied that the reaction between TMH and perchloric acid proceeds stoichiometrically in the ratio 1:1 in the range studied.

Method optimization and repeatability precision. In both the methods, the optimum amount of mercuric acetate required was studied by varying its amount and fixing the drug amount constant, followed by the measurement of the stoichometric amount of drug found in each case. It was found that a 3 mL of 3 % $Hg(OAc)_2$ was sufficient for complete replacement of chloride in the drug by acetate, and the same amount was used through out the investigation.

Intra-day and inter-day accuracy and Precision. The precision of the methods was evaluated in terms of intermediate precision (intra-day and inter-day). Three different amounts of TMH within the range of study in each method were analyzed in seven and five replicates in method A and method B, respectively, during the same day (intra-day precision) and five consecutive days (inter-day precision). For inter-day precision, each day analysis was performed in triplicate and pooled-standard deviation was calculated. The RSD values of intra-day and inter-day studies for TMH showed that the precision of the methods was good (Table 1). The accuracy of the methods was determined by the percent mean deviation from known amount (Table 1).

Table 1 Intra-day and inter-day accuracy and precision data.

Method	TMH taken, mg	Intra-day accuracy and precision			Inter-day accuracy and precision		
		TMH found, mg	RE, %	RSD, %	TMH found, mg	RE, %	RSD, %
Visual titrimetry, (n=7)	5.0	5.05	1.00	1.10	5.06	1.20	1.56
	10.0	10.17	1.70	0.44	9.90	1.00	1.23
	15.0	15.25	1.66	0.60	14.89	0.73	1.20
Potentiometric titrimetry (n=5)	5.0	5.08	1.66	0.95	4.96	0.80	0.98
	10.0	10.10	0.98	0.86	10.05	0.50	1.06
	15.0	15.11	0.73	0.88	15.10	0.67	1.56

RE. relative error, RSD. relative standard deviation

*Robustness and ruggedness of methods.*The robustness of the methods was evaluated by making small incremental changes in volume of $Hg(OAc)_2$ and the effect of the changes was studied by calculating the mg TMH found. The changes had negligible influence on the results as revealed by small intermediate precision values expressed as % RSD (< 3%).

Method ruggedness was expressed as the RSD of the same procedure applied by four different analysts, as well as using four different burettes. The inter-analysts RSD were within 2.22%, whereas the inter-burettes RSD for the same TMH amount were less than 2.63%, suggesting that the developed method was rugged (Table 2).

Table 2 Method robustness and ruggedness expressed as intermediate precision (% RSD).

Method	TMH taken, mg	Robustness	Ruggedness	
		Volume of $Hg(OAc)_2$*	Inter-analysts (%RSD), (n=4)	Inter-instruments (%RSD), (n=4)
Visual titrimetry	5.0	1.42	2.22	2.36
	10.0	1.20	2.10	2.63
	15.0	2.10	1.99	2.52
Potentiometric titrimetry	5.0	1.20	1.22	2.13
	10.0	1.56	1.36	1.76
	15.0	1.98	1.56	1.59

* The volume of $Hg(OAc)_2$ varied were 2.8, 3.0 and 3.2 mL

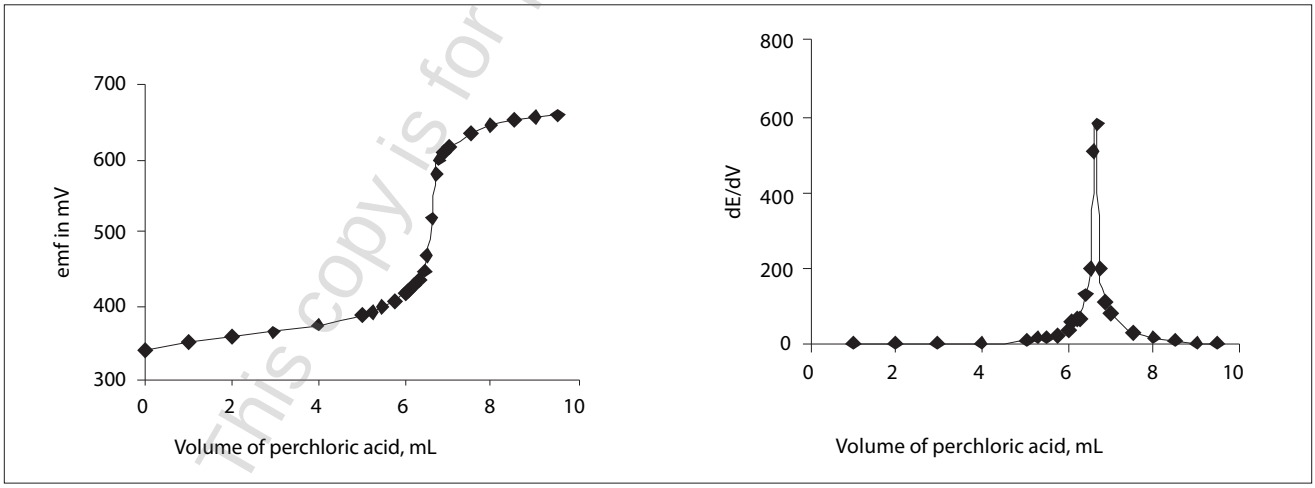


Figure 1 Potentiometric titration curves for 20 mg TMH *Vs* 0.01 M $HClO_4$.

APPLICATION

The described titrimetric procedures were successfully applied for the determination of TMH in its pharmaceutical formulations. The same batch tablets were also assayed by the reference method [20] for comparison. The reference method consisted of the UV-spectrophotometric measurement of TMH in water at 271 nm. The results obtained by the proposed methods agree well with those of the reference method and with the label claim. The results were also compared statistically by Student's t-test for accuracy, and by a variance F-test for precision [27], with those of the reference method at 95 % confidence level as summarized in Table 3. The results showed that the calculated t-and F-values did not exceed the tabulated values, inferring that the proposed methods are as accurate and precise as the reference method.

Table 3 Results of assay in tablets and comparison with the reference method.

Brand name	Label claim, mg/ tablet	Found* (Percent of label claim ± SD)		
		Official method	Proposed methods	
			Visual titrimetry	Potentiometric titrimetry
Tramazac 100	100	98.64±0.62	100.3±1.28 t= 2.76 F= 4.26	99.87±1.15 t= 2.19 F= 3.44
Trambax 50	50	97.33±0.76	98.16±1.04 t= 1.46 F= 1.87	97.58±0.92 t= 0.47 F= 1.46
Cemadol 100	100	101.8±0.54	103.2±1.16 t= 2.60 F= 4.61	100.8±1.08 t= 1.95 F= 4.00

* Average of five determinations. Tabulated t value at the 95% confidence level is 2.77
Tabulated F value at the 95% confidence level is 6.39

Recovery Study. Accuracy and the reliability of the methods were further ascertained by performing recovery experiments. To a fixed amount of drug in formulation (pre-analyzed): pure drug at three different levels was added, and the total was found by the proposed methods. Each test was repeated three times. The results (Table 4) show that recoveries were in the range 98.36-107.3 %, indicating that commonly added excipients to the tablets did not interfere in the determination.

CONCLUSIONS

Two rapid and simple acid-base titrimetric procedures in non-aqueous medium have been developed, optimized and validated for the quantitative estimation of TMH. Acceptable assay precision and accuracy were obtained over a wide range of amounts (mg) in both methods. Assay results indicated that there was no interference from the excipients, additives and diluents commonly added to dosage forms. The methods could serve as useful tool for the rapid analysis of bulk drug and formulations, and thus show great potential as an alternative to expensive instrumental techniques.

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REFERENCES

1. Grosa G, Grosso ED, Russo R, Allegrone G: Simultaneous, stability indicating, HPLC-DAD determination of guaifenesin and methyl and propyl-parabens in cough syrup. *J Pharm Biomed Anal* 2006, **41**, 798-803.
2. Lintz W, Barth H, Osterloh G, Schmidt BE: Pharmacokinetics of tramadol and bioavailability of enteral tramadol formulations. 3rd Communication: suppositories. *Drug Res* 1998, **48**, 889-899.
3. 'Tramadol, Ibuprofen', **in:** *British Pharmacopoeia,* HM Stationary Office, London 2004.
4. Aysel K, Yucel K: Determination of tramadol hydrochloride in ampoule dosage forms by using UV spectrophotometric and HPLC-DAD methods in methanol and water media. *IL Farmaco* 2005, **60**, 163-169.
5. Kartinasari WF, Palupi T, Indrayanto G: hplc determination and validation of tramadol hydrochloride in capsules. *J Liq Chromatogr Rel Tech* 2005, **27**, 737-744.
6. Zaghloul IY, Radwan MA: High performance liquid chromatographic determination of tramadol in pharmaceutical dosage forms. *J Liq Chromatogr Rel Tech* 1997, **20**, 779-787.
7. Zhong YL: Determination of the content of tramadol hydrochloride sustained-release tablets. *Yaowu Fenxi Zazhi* 1997, **17**, 279-280.
8. Krzek J, Starek M: Quality assessment for tramadol in pharmaceutical preparations with thin layer chromatography and densitometry. *Biomed Chromatogr* 2004, **18**, 589-599.
9. Pospisilova M, Polasek M, Jokl V: Determination of tramadol in various dosage forms by capillary isotachophoresis. *J Pharm Biomed Anal* 1998, **18**, 777-783.
10. Ganjali MR, Memari Z, Faridbod F, Dinarvand R, Norouzi P: Sm^{3+} potentiometric membrane sensor as a probe for determination of some pharmaceutics. *Electroanalysis,* New York 2008, **20**, 2663-2670.

Table 4 Results of recovery study using standard addition method.

Tablet studied	Visual titrimetry				Potentiometric titrimetry			
	TMH in tablet extract, mg	Pure TMH added, mg	Total TMH found, mg	Pure TMH recovered* %	TMH in tablet extract, mg	Pure TMH added, mg	Total TMH found, mg	Pure TMH recovered* %
Tramazac 100	8.02	4.00	12.22	105.10±1.24	4.00	4.00	8.14	103.50±1.26
	8.02	8.00	16.60	107.30±0.96	4.00	8.00	12.13	101.62±0.85
	8.02	12.00	20.33	102.60±1.12	4.00	12.00	16.52	104.33±0.97
Trambax 50	3.93	4.00	7.86	98.25±1.34	3.90	4.00	7.88	99.50±0.78
	3.93	8.00	12.07	101.75±0.86	3.90	8.00	12.26	104.50±0.86
	3.93	12.00	16.34	103.40±0.72	3.90	12.00	16.10	101.67±1.03

*Mean value of three determinations

11. Wu YJ, Zhang JH, Zhao KL, Zhang ZP: Preparation and application of an ion-selective electrode by tramadol hydrochloride. *Fenxi Shiyanshi* 2005, **24**, 49-51
12. Huang CL, Xiu R, Liu ZY, Li YC, Meng CJ: Preparation and application of a doubly- coated membrane tramadol ion-selective electrode based on Ag/AgCl wir**.** *Fenxi Kexue Xuebao* 2005, **21**, 48-50.
13. Huang CL, Xiu R, Dong QX, Chen XZ, You HQ: Preparation of a PVC membrane tramadol ion-selective electrode based on gel quinhydrone internal reference electrode. *Fenxi Ceshi Xuebao* 2005, **24**, 35-38.
14. Wang M, Long YL, Li DH, Zhou WB, Zhao GZ, Zhang Z: Preparation of an ofloxacin-poly(vinyl chloride) membrane and tramadol-selective electrodes and their application. *Fenxi Huàxué Fenxi* 1997, **25**, 448-451.
15. Hopkala H, Misztal G, Wieczorek A: Tramadol selective PVC membrane electrodes and their analytical application**.** *Die Pharmazie* 1998, **53**, 869-871.
16. Yue JL, Yu QH, Li DH, Zhao GZ, Shi W, An LR: Preparation and application of a polyvinyl chloride-membrane tramadol-selective electrode. *Fenxi Huàxué* 2000, **28**, 84-86.
17. Norouzi P, Dinarvand R, Ganjali MR, Meibodi ASE: Application of adsorptive stripping voltammetry for the nano-level detection of tramadol in biological fluids and tablets using fast fourier transform continuous cyclic voltammetry at an Au microelectrode in a flowing system. *Anal Lett* 2007, **40**, 2252-2270.
18. Garrido EMPJ, Garrido JMPJ, Borges F, Delerue-Matos C: Development of electrochemical methods for determination of tramadol - analytical application to pharmaceutical dosage forms. *J Pharm Biomed Anal* 2003, **32**, 975-981.
19. Yang YZ, Lu YB: Ultra-violet-spectrophotometric determination of tramadol hydrochloride in injection solution. *Yaowu Fenxi Zazhi* 1996, **16**, 132-132.
20. Mang FW, Liu W, Yang JN: Ultra-violet spectrophotometric analysis of tramadol hydrochloride injections. *Yaowu Fenxi Zazhi* 1994, **14**, 42-42.
21. Zhang JK, Li JG, Tu YF: Flow injection chemiluminescence determination of tramadol hydrochloride. *Fenxi Kexue Xuebao* 2009, **25**, 173-176.
22. Abdellatef HE, El-Henawee MM, El-Sayed HM, Ayad MM: Spectrophotometric and spectrofluorimetric methods for analysis of tramadol, acebutolol and dothiepin in pharmaceutical preparations. Spectrochim Acta Part A. **In:** *Mol Biomol Spectro* 2006, **65**, 1087-1092.
23. Abdellatef HE: Kinetic spectrophotometric determination of tramadol hydrochloride in pharmaceutical formulation. *J Pharm Biomed Anal* 2002, **29**, 835-842.
24. Sadhana RJ Trivedi Priti D: Assay of tramadol hydrochloride by spectrophotometry. *Indian Drugs* 2001, **38**, 100-101.
25. Jiri Kucharski RN, Safarik LA, *Text Book of 'Titrations In Non-Aqueous Solvents'*. Elsevier, Amsterdam-London-NewYork 1965, 94.
26. Ashutosh Kar, Pharm. *Drug Anal* (2nd ed.), New Age International Publisher, New Delhi 2005, 111.
27. Inczedy J, Lengyel T, Ure AM, *IUPAC Compendium of Analytical Nomenclature: Definitive Rules*, Blackwell Science Inc, Boston 1998.