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BROMATOMATRIC ASSAY OF GATIFLOXACIN IN PHARMACEUTICALS

Three new, simple, and cost-effective visible spectrophotometric methods are proposed for determination of gatifloxacin (GTF) using bromate-bromide mixture, and three dyes, methyl orange, indigocarmine and thymol blue, as reagents. The methods engross the addition of a known excess of bromate-bromide mixture to GTF in hydrochloric acid medium followed by determination of residual bromine by reacting with a fixed amount of either methyl orange and measuring the absorbance at 520 nm (method A) or indigo carmine and measuring the absorbance at 610 nm (method B) or thymol blue and measuring the absorbance at 550 nm (method C). In all the methods, the amount of bromine reacted corresponds to the amount of GTF, and the absorbance is found to increase linearly with the concentration of GTF. Under the optimum conditions, GTF could be assayed in the concentration range 0.25-1.5, 0.5-6.0, and 0.5-10 μg/mL by method A, method B and method C, respectively. The apparent molar absorptivities are calculated to be $1.6x10^5$, $4.0x10^4$ and $3.2x10^4$ L mol^{-1} cm^{-1} for the method A, method B and method C, respectively, and the corresponding Sandell sensitivity values are 0.0025, 0.010 and 0.012 μg/cm^2. The intra-day and inter-day precision, and the accuracy of the methods were evaluated as per the current ICH guidelines. The methods were successfully applied to the determination of GTF in pharmaceutical preparations without the interference from any of the pharmaceutical adjuvants.

Key words: gatifloxacin; assay; spectrophotometry; bromate-bromide; formulations.

Gatifloxacin(GTF) is a synthetically derived, broad spectrum fluoroquinolone designed for both oral and intravenous administration (Fig. 1). Chemically, it is (±)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(3--methyl-1-piperazinyl)-4-oxo-3-quinoline carboxylic acid sesqui hydrate [1]. It is indicated for acute pyelonephritis, acute bacterial exacerbation of chronic bronchitis and complicated UTI.

Various techniques have been used for the determination of GTF in body fluids and pharmaceuticals. High performance liquid chromatography (HPLC) has been applied for the determination of the drug in plasma [2,3], serum [4], and serum and urine [5]. The drug in urine and serum has also been quantified by spectrofluorimetry [6]. There is only one report on the application of HPLC for the assay of GTF in bulk drug and dosage forms. A non-aqueous titration procedure [7] has recently been described for the assay of a drug in pharmaceutical formulations using perchloric acid as titrant. Very recently, Salgado *et al.* [8] have reported a microbiological assay for GTF in pharmaceutical formulations. Several UV-spectrophotometric [9-13] and AAS, conductometric and colorimetric [14] procedures employing different media have also been reported for the assay in single as well as combined dosage forms.

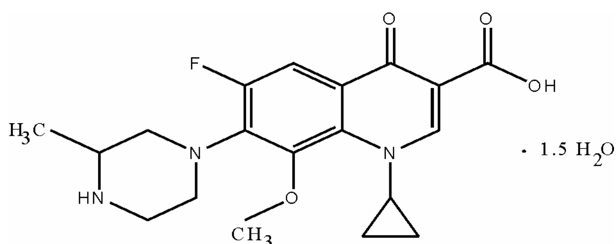

Fig. 1. The structure of Gatifloxacin sesquihydrate.

Because of simplicity, cost effectiveness, sensitivity, selectivity and fair accuracy and precision, visible spectrophotometry has remained competitive in an era of chromatographic techniques for pharmaceutical ana-

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lysis. Many visible spectrophotometric methods based on different reaction schemes are found in the literature for the assay of GTF. In the method reported by Dhachinamoorthy *et al.* [15] ferric ferricyanide was reduced by GTF, and the blue chromogen formed was measured forming the basis of the assay. The yellow-orange chromogen formed when GTF was treated with cerium (IV) was used by Devala and Babu [16] for the determination of the drug in 40-160 μg/mL range in dosage forms. Two methods, one based on redox-complexation reaction involving chromium (VI) and sym-diphenylcarbazide and the other on Mannich reaction, have recently been reported by Saraswathi *et al.* [17]. There are two reports [18,19] on the use of N-bromosuccinimide (NBS) as an oxidimetric reagent for the estimation of GTF. The methods are based on the determination of unreacted NBS with celestine blue or by charge transfer reaction involving metol and sulphanilamide. A similar method, but using chloramine-T and gallocyanine [19] as reagents, is also found in the article. Three sensitive methods [20] based on chloroform extractable ion-association complexes formed by GTF with wool fast blue BL, Tropaeolin ooo or bromophenol blue are also found in the literature. Amin *et al.* [21] have used the methods based on the formation of yellow ion-pair complexes between the basic nitrogen of the drug and three sulphonphthalein acid dyes, namely; bromocresol green (BCG), bromocresol purple (BCP), bromophenol blue (BPB) and bromothymol blue (BTB) in phthalate buffer pH 3.0, 3.4 and 3.2, using BCG, BCP and (BPB or BTB), respecttively. The formed complexes were extracted with chloroform and measured at 415, 417, 412 and 414 nm for BCG, BPB, BCP and BTB, respectively. Gouda *et al.* [22] have reported the methods based on the reaction of gatifloxacin as n-electron donor with 7,7,8,8-tetracyanoquinodimethane (TCNQ); 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ); chloranilic acid (CLA) and *p*-chloranil (CL) as π-acceptors to give highly colored complex species. The colored products are quantified spectrophotometrically at 460, 841, 530 and 545 nm for DDQ, TCNQ, CLA and CL, respectively.

The present investigation aims to develop sensitive and cost-effective methods for the determination of GTF in pure form and in dosage forms using visible spectrophotometry. The methods utilize the bromate-bromide mixture as an oxidimetric/brominating reagent which has successfully been used for the sensitive spectrophotometric determination of many bioactive substances [23-33]. The proposed methods have the advantages of speed and simplicity besides being accurate and precise, and can be adopted by the pharmaceutical laboratories for the industrial quality control.

EXPERIMENTAL

Reagents and materials

All chemicals used were of an analytical reagent grade, and distilled water was used to prepare all the solutions. A stock standard solution equivalent to 1000 μg/mL $KBrO_3$ containing a large excess of KBr was prepared by dissolving the accurately weighed 100 mg of $KBrO_3$ (Sarabhai M. chemicals, Baroda, India) and 1.0 g of KBr (S.d. Fine Chem. Ltd., Mumbai, India) in water and diluting to 100 mL in a volumetric flask. The above solution was diluted appropriately with water to get 10, 30 and 60 μg/mL concentrations for method A, B and C, respectively. To prepare 50 μg/mL methyl orange for method A, a 500 μg/mL dye solution was first prepared by dissolving the accurately weighed 58.8 mg of dye (S.d. Fine Chem., Mumbai, India, 85 % dye content) in water, and diluting it to 100 mL in a calibrated flask and filtered it by using glass wool. It was further diluted to obtain a required concentration. For method B, a 1000 μg/mL stock standard solution was prepared by dissolving the accurately weighed 112 mg of dye (S.d. Fine Chem., Mumbai, India, 90 % dye content) in water and diluting it to the volume in a 100 mL calibrated flask. The solution was then diluted 5-fold to get the working concentration of 200 μg/mL. For method C, a 200 μg/mL standard solution was first prepared by dissolving the accurately weighed 20 mg of dye (Loba Chem., Mumbai, India, 100% dye content) in water and diluting to the volume in a 100 mL calibrated flask. Hydrochloric acid (5 mol/l) was prepared by diluting 43 mL of concentrated acid (S.d. Fine Chem. Ltd., Mumbai, Sp gr 1.18) to 100 mL with water. Pharmaceutical grade GTF, certified to be 99.85 % pure was procured from Cipla India Ltd, Mumbai, India, and was used as received. A 1 mg/mL solution of GTF was prepared by dissolving accurately weighed 100 mg of pure drug in 25 mL water with the aid of heat to get clear solution, followed by diluting to 100 mL with water in a calibrated flask. This stock solution (1000 μg/mL) was diluted with water to get working concentrations of 5, 20 and 25 μg/mL GTF for methods A, B and C, respectively.

Methods

Method using methyl orange (method A)

Aliquots of pure GTF solution (0.5 to 3.0 mL; 5 μg/mL) were transferred into a series of 10 mL calibrated flasks and the total volume was adjusted to 3.0 mL with water. To each flask, 2mL of 5 mol/L hydrochloric acid were added followed by 1mL of bromate-bromide mixture (10 μg/mL). The content was mixed well and the flasks were set aside for 10 min with occasional shaking. Finally, 1 mL of 50 μg/mL methyl or-

ange solution was added to each flask, diluted to the mark with water, and the absorbance of the solution was measured at 520 nm against a reagent blank after 10 min.

Method using indigo carmine (method B)

Varying aliquots (0.25-3.0 mL) of standard 20 μg/mL GTF solution were measured accurately and delivered into a series of 10 mL calibrated flasks and the total volume was brought to 3.0 mL with water. To each flask, 2 mL of 5 mol/L hydrochloric acid and 1.5 mL of 30 μg/mL bromate-bromide mixture were added by means of a micro burette; the flasks were let stand for 15 min with occasional shaking. Then, 1 mL of 200 μg/mL indigo carmine solution was added to each flask; the volume was adjusted to the mark with water and mixed well. The absorbance of each solution was measured at 610 nm against a reagent blank after 10 min.

Method using thymol blue (method C)

Varying aliquots (0.2-4.0 mL) of the standard 25 μg/mL GTF solution were measured accurately and delivered into a series of 10 mL calibrated flasks and the total volume was brought to 4.0 mL with water. To each flask, 1mL of 5 mol/L hydrochloric acid and 60 μg/mL bromate-bromide mixture were added by means of a micro burette; the flasks were let stand for 5 min with occasional shaking. Then, 1 mL of 200 μg/mL thymol blue solution was added to each flask, the volume was adjusted to the mark with water and mixed well. The absorbance of each solution was measured at 550 nm against a reagent blank after 10 min.

In all the methods, the concentration of the unknown was read from the calibration graph or computed from the regression equation derived from the Beer's law data.

Assay procedure for formulations

An amount of a finely ground tablet powder equivalent to 100 mg of GTF was accurately weighed into a beaker, 50 mL water was added and stirred for 20 min and warmed. Then, the content was quantitatively transferred into a 100 mL calibrated flask, the beaker was washed with water, the washings were transferred to the flask and the volume was made up to the mark with water, mixed well, and filtered using a Whatman No 42 filter paper. First 10-mL portion of the filtrate was discarded and a suitable aliquot of the subsequent portion (1000 μg/mL GTF) was diluted appropriately to get 5, 20 and 25 μg/mL concentrations for the analysis by methods A, B and C, respecttively.

Selectivity testing

Selectivity test was performed by applying the proposed methods to the determination of GTF in a synthetic mixture consisting of: GTF (100 mg), talc (250 mg), starch (300 mg), lactose (30 mg), calcium gluconate (50 mg), calcium dihydrogenorthophosphate (20 mg), sodium alginate (70 mg) and magnesium stearate (100 mg), in the ratio of 1:2.5:3.0:0.3:0.5:0.2:0.7:1, GTF was extracted with three 20-mL portions of water, with heating, and filtered. The filtrate was washed with water; the filtrate and the washings were collected in a 100 ml calibrated flask and diluted to volume with water and mixed well. A convenient aliquot of the extract was subjected to the analysis as stated earlier.

RESULTS AND DISCUSSION

Optimization of experimental conditions

The proposed spectrophotometric methods are indirect and are based on the determination of the residual bromine (*in situ* generated) after allowing the reaction between GTF and a measured amount of bromine to be complete. The surplus bromine was determined by reacting it with a fixed amount of either methyl orange or indigo carmine or thymol blue dye. The methods make use of a bleaching action of bromine on the dyes, the discoloration being caused by the oxidative destruction of the dyes.

When added in increasing amounts to a fixed amount of *in situ* generated bromine, GTF consumes the latter proportionately and there occurs a concomitant fall in the amount of bromine. When a fixed amount of dye is added to decreasing amounts of bromine the result is a concomitant increase in the concentration of dye. Consequently, a proportional increase in the absorbance at the respective λ_{max} is observed with increasing concentration of GTF.

Preliminary experiments were performed to fix the upper limits of the dyes that could be determined spectrophotometrically, and these were found to be 5, 20 and 20 μg/mL for methyl orange , indigo carmine and thymol blue, respectively. In method A, a bromate concentration of 1.0 μg/mL was found to irreversibly destroy the red colour of 5 μg/mL methyl orange, whereas in method B, 4.5 μg/mL bromate was required to bleach the blue colour due to 20 μg/mL indigo carmine and in method C, a bromate concentration of 6.0 μg/mL was found to irreversibly destroy the blue colour of 20 μg/mL thymol blue. Hence, different amounts of GTF were reacted with 1 mL of 10 μg/mL bromate in method A, 1.5 mL of 30 μg/mL bromate in method B, and 1.0 mL of 60 μg/mL bromate in method C, followed by the determination of the residual bromine as describeed, under the respective procedures.

For both steps, *i.e.*, bromination of the drug and bleaching of the dye by bromine, hydrochloric acid

medium was found to be ideal. Two mL of 5 mol/L hydrochloric acid for methods A and B, and 1 mL of 5 mol/L HCl for method C in a total volume of ca. 3-4 mL was adequate for the bromination step, which was complete in 10, 15 and 5 min in methods A, B and C, respectively, and the same quantity of acid was employed for the estimation of the dye. The contact time is not critical and any delay up to 30 min had no effect on the absorbance.

Analytical data

A linear correlation was found between the absorbance at λ_{max} and the concentration of GTF.

The graphs showed a negligible intercept and are described by the equation:

$Y = a + bX$

where Y is the absorbance of a 1-cm layer of the solution, a is the intercept, b is the slope and X is the concentration in μg/mL. The regression analysis of the Beer's law data using the method of least squares was made to evaluate the slope (b), the intercept (a) and the correlation coefficient (r) for each system and the values are presented in Table 1. The optical characteristics such as Beer's law limits, molar absorptivity and Sandell sensitivity values of the methods are also given in Table 1. The limits of detection (*LOD*) and quantitation (*LOQ*) calculated according to ICH guidelines [33] are also presented in Table 1 and reveal the very high sensitivity of the proposed methods.

Method Validation

Accuracy and precision

To evaluate the accuracy and precision of the methods, a pure drug solution at three different levels (within the working limits) was analysed, each determination being repeated seven times. The relative error (%) and relative standard deviation (%) were less than 2.0 and indicate the high accuracy and precision of the methods (Table 2). For a better picture of reproducibility on a day-to-day basis, a series of experiments were performed in which a standard drug solution at three different levels was determined each day for five days with all solutions being prepared afresh each day. The day-to-day relative standard deviation values were in the range of 2.5-3.5 % and represent the best appraisal of the methods in routine use.

Table 1. Analytical and regression parameters of the proposed methods

Parameter	Method A	Method B	Method C
λ_{max}, nm	520	610	550
Beer's law limits, μg/mL	0.25-1.50	0.5-6.0	0.5-10.0
Molar absorptivity, L mol^{-1} cm^{-1}	1.6×10^5	4.0×10^4	3.2×10^4
Sandell sensitivity, μg/cm^2	0.0025	0.010	0.012
LOD, μg/mL	0.02	0.06	0.09
LOQ, μg/mL	0.05	0.20	0.28
Intercept (a)	-0.004	-0.023	-0.010
Slope (b)	0.3989	0.1086	0.0836
Correlation coefficient (r)	0.9996	0.9989	0.9948
S_a*	0.0061	0.0035	0.0034
S_b**	0.0058	0.0008	0.0060

*Standard deviation of intercept; **standard deviation of slope

Table 2. The evaluation of accuracy and precision (RE: Relative error; SD: Standard deviation; SEM: Standard error of mean; RSD: Relative standard deviation; ROE: Range of error)

Method	GTF taken,μg /mL	GTF found,* μg/mL	Range, μg/mL	*RE*, %	*SD*, μg/mL	*SEM*, μg/mL	*RSD*, %	*ROE*, %
A	0.5	0.49	0.01	1.83	0.004	0.002	0.85	±0.85
	1.0	0.98	0.04	1.57	0.018	0.007	1.82	±1.82
	1.5	1.48	0.05	1.67	0.017	0.006	1.15	±1.15
B	2.0	1.98	0.05	1.15	0.02	0.006	0.82	±0.82
	4.0	3.97	0.17	0.82	0.06	0.021	1.40	±1.40
	6.0	5.92	0.25	1.38	0.08	0.031	1.37	±1.37
C	3.0	2.96	0.14	1.51	0.05	0.019	1.67	±1.67
	5.0	4.93	0.11	1.47	0.04	0.015	0.81	±0.81
	7.0	6.87	0.10	1.86	0.04	0.014	0.52	±0.52

*Mean value of seven determinations; **at the 95 % confidence level for 6 degrees of freedom

Application to analysis of commercial samples

In order to check the validity of the proposed methods, GTF was determined in some commercial formulations. Table 3 gives the results of the determination from which it is clear that there is close agreement between the results obtained by the proposed methods and the label claim. The results were also compared statistically by a Student's t-test for accuracy and variance ratio F-test for precision with those of the reference method [9] at 95 % confidence level. The calculated t and F values (Table 3) did not exceed the tabulated values (t = 2.77 and F = 6.39, except in a couple of instances) for four degrees of freedom, which indicate that there was no significant difference between the proposed methods and the reference method in respect to accuracy and precision.

Table 3. The results of determination of gatifloxacin in formulations and a statistical comparison with the reference method

Tablet brand name*	Nominal amount, mg	% found** ± SD			
		Reference method	Method A	Method B	Method C
GAITY[a]	400	100.3±0.51	99.5±0.69 t = 2.11*** F = 1.83 ****	101.3±0.56 t = 2.95 F = 1.21	99.5±0.44 t = 2.66 F = 1.34
GATIQUIN[b]	200	99.6±0.65	98.6±1.01 t = 1.90 F = 2.41	101.6±1.02 t = 3.78 F=2.46	100.2±1.06 t = 1.11 F=2.66
G-CEBRAN[c]	400	101.3±0.62	99.8±1.32 t = 2.44 F = 4.53	101.9±1.33 t = 0.97 F = 4.60	100.3±1.06 t = 1.88 F = 2.92

*Marketed by: a: Reddy's Ltd., b) Cipla Ltd.and c) Blue Cross Ltd.; **mean value of five determinations; ***tabulated t value at 95% confidence level is 2.77; ****tabulated F value at 95% confidence level is 6.39

Table 4. The results of recovery experiments by the standard addition method

Tablets studied	Method A				Method B				Method C			
	GTF in tablet, μg	Pure GTF added, μg	Total found, μg	Pure drug recovered* %	GTF in tablet, μg	Pure GTF added, μg	Total found, μg	Pure drug recovered* %	GTF in tablet, μg	Pure GTF added, μg	Total found, μg	Pure drug recovered* %
GAITY	3.98	2	6.01	101.3	20.26	10	30.58	103.2	24.88	10	34.64	97.56
	3.98	4	8.07	102.2	20.26	20	40.16	99.5	24.88	30	56.26	104.6
	3.98	6	9.91	98.75	20.26	30	50.35	100.3	24.88	50	75.03	100.3
G-CEBRAN	3.99	2	5.98	99.6	20.38	10	30.59	102.1	25.08	10	35.01	99.28
	3.99	4	8.20	105.3	20.38	20	40.02	98.2	25.08	30	54.38	97.65
	3.99	6	10.07	101.3	20.38	30	50.44	100.2	25.08	50	77.33	104.5

*Mean value of three determinations

The accuracy and validity of the proposed methods were further ascertained by performing recovery studies. A pre-analysed tablet powder was spiked with pure GTF at three different levels and the total was found by the proposed methods. Each determination was repeated three times. The recovery of the pure drug added was quantitative and revealed that co-formulated substances such as talc, starch, gelatin, gum acacia, calcium carbonate, calcium gluconate, calcium dihydrogen orthophosphate, sodium alginate and magnesium stearate did not interfere in the determination. The results of the recovery study are compiled in Table 4.

CONCLUSIONS

Three useful micro methods for the determination of GTF have been developed and validated. The methods are simple and rapid taking not more than 20-25 min for the assay. Among the three methods, the method A is more sensitive than methods B and C. Compared to the reported method [32], the present methods are highly sensitive and, in fact, the method A (ε =1 .6×10^5 L mol^{-1} cm^{-1}) is the most sensitive ever reported for GTF. Also, the proposed methods are more sensitive than the existing UV and HPLC methods, and are free from such experimental variables as heating or the extraction step. The methods rely on the use of simple and cheap chemicals and techniques but provide sensitivity comparable to

that achieved by a sophisticated and expensive technique like HPLC. Thus, they can be used as alternatives for the rapid and routine determination of a bulk sample and tablets.

REFERENCES

[1] N. Douglas, Pharmacotherapy **21** (2001) 35

[2] H. Liang, M.B. Kays, K.M. Sowinski, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. **772** (2002) 53

[3] S. Al-Dgither, S.N. Alvi, M.M. Hammami, J. Pharm. Biomed. Anal. **41** (2006) 251

[4] H.A. Nguyen, J. Grellet, B.B. Ba Claudine Quentin, Marie-Claude Saux, J. Chromatogr. B. **810** (2004) 77

[5] B.R. Overholser, M.B. Kays, K.M. Sowinski, J. Chromatogr. B. Anal. Technol. Biomed. Life Sci. **798** (2003) 167

[6] J.A. Ocana, F.J. Barragan, M. Callejon, J. Pharm. Biomed. Anal. **37** (2005) 327

[7] R.N. Marona Herida, C.G.O. Lopes Cristiani, G. Cardoso Simone, Acta Farmaceutica Bonaerense **22** (2003) 339

[8] H.R.N. Salgado, C.C.G.O. Lopes, M.B.B. Lucchesi, J. Pharm. Biomed. Anal. **40** (2006) 443

[9] K. Venugopal, R.N. Saha, IL Farmaco **60** (2005) 906

[10] P.U. Patel, B.N. Suhagia, C.N. Patel, M.M. Patel, C. Patel Gayatri, M. Patel Geeta, Indian J Pharm. Sci. **67** (2005) 356

[11] Wang Xiao-Ling, Li Dong, Nie Zhong-Yue, Yaoxue Fuwu Yu Yanjiu **5** (2005) 57

[12] H.R.N. Salgado, C.L.C.G. Oliveira, Pharmazie **60** (2005) 263

[13] Xu Qin, Qin Xuelian, Deng Lidong, Guangdong Yaoxueyuan Xuebao **19** (2003) 234

[14] S.M. Al-Ghannam, Spectrochim. Acta Part A. **69** (2008) 1188

[15] D. D. Suvakanta, G. Mariappan, K. Sangeetha, Pharma Review **2** (2004) 120

[16] G. Devala, B.I. Sudheer, Indian Pharmacist. **3** (2004) 78

[17] Thangavel Neelaveni, Raman Saraswati, S. Angalaparameswari , K.P. Beena, Int. J. Chem. Sci. **3** (2005) 263

[18] K.V. Siva Prasad, G. Prabhakar, S.V.M. Mohan Rao, K. Satish kumar, Haritha, Acta Ciencia Indica **29** (2003) 163

[19] K.V. Siva Prasada Rao, C. Srinivasulu, P. Nagaraju, S.R. Sagineedu, Prabhakar, Oriental J. Chemistry **19** (2003) 583

[20] K.V. Siva Prasad, G. Prabhakar, S.V.M. Mohan Rao, M. Sandhya, Jagganath, Asian J. Chemistry **15** (2003) 1170

[21] A.S. Amin, A. A. F. Gouda, R. El-Sheikh, Z. Faten, Spectrochim. Acta **67** (2007) 1306

[22] A.A.F. Gouda, R. El-Sheikh, A.S. Amin, Chem Pharm Bull. **56** (2008) 34

[23] K. Basavaiah, H.C. Prameela, Science Asia **29** (2003) 147

[24] K. Basavaiah, H.C. Prameela , Anal. Bioanal. Chem. **376** (2003) 879

[25] K. Basavaiah, U. Chandrashekar, Acta Ciencia Indica Chem. **29** (2003) 25

[26] K. Basavaiah, P. Nagegowda, IL Farmaco **59** (2004) 147

[27] K. Basavaiah, P. Nagegowda, Oxid. Commun. **27** (2004) 186

[28] K. Basavaiah, H.C. Prameela, Indian J. Pharm. Sci. **67** (2004) 883

[29] K. Basavaiah, Indian J. Chem. Technol. **12** (2005) 25

[30] K. Basavaiah, P. Nagegowda, J. Braz. Chem. Soc. **16** (2005) 821

[31] K. Basavaiah, B.C. Somashekar, Indian J. Chem. Technol. **12** (2006) 316

[32] K. Basavaiah, U.R. Anilkumar, E-J. Chem. **4** (2007) 154

[33] "*Validation of Analytical Procedures*; Methodology, International Conference on Harmonization (ICH), Text and Methodology Q2(R 1), Complementary Guideline on Methodology dated 06 November 1996, incorporated in November 2005, London.