RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2006.32.403

ScienceAsia 32 (2006): **403-409**

Spectrophotometric and Titrimetric Determination of Ciprofloxacin Based on Reaction with Cerium (IV) Sulphate

Kanakapura Basavaiah[1]*, Paregowda Nagegowda[1], Bankavadi Chikkaswamy Somashekar[1]and Veeraiah Ramakrishna[2]

[1] Department of Chemistry, University of Mysore, Manasagangothri, Mysore – 570 006, Karnataka, India.
[2] Department of Pharmaceutical Chemistry, Govt. College of Pharmacy, Bangalore-560027, Karnataka. India.

* Corresponding author, E-mail: basavaiahk@yahoo.co.in

Received 24 Aug 2005
Accepted 16 Jun 2006

ABSTRACT: One titrimetric and two spectrophotometric methods are described for the determination of ciprofloxacin in bulk drug and in formulations using cerium (IV) sulphate as the oxidimetric agent and methyl orange and indigo carmine as chromogenic agents. In titrimetry (method A), ciprofloxacin is treated with a measured excess of cerium (IV) sulphate in acid medium and the unreacted oxidant is back titrated with standard ammonium ferrous sulphate using ferroin indicator. In spectrophotometric methods, ciprofloxacin is treated with a known excess of cerium (IV) sulphate and the residual oxidant is determined by treating with a fixed amount of either methyl orange, and measuring the absorbance at 520 nm (Method B) or indigo carmine, and measuring the absorbance at 610 nm (Method C). In all the three methods, the amount of cerium (IV) sulphate reacted corresponds to the amount of ciprofloxacin. Titrimetry is applicable over 2-12 mg range and in the spectrophotometric methods, calibration curves are linear over the concentration ranges of 0.5-3.5 μg ml^{-1} (method B) and 1.0-7.0 μg ml^{-1} (method C). The methods were satisfactorily applied to the determination of ciprofloxacin in tablet and injection formulations and no interferences from excipients were noticed.

KEYWORDS: Ciprofloxacin; titrimetry; spectrophotometry; cerium(IV) sulphate; two dyes; dosage forms; redox reaction.

INTRODUCTION

Ciprofloxacin (CPF) is, chemically, 1-cyclopropyl-6-fluoro-1,4- dihydro-4- oxo- 7 (piperazin-1-yl) quinoline-3-carboxylic acid (Fig.1) and belongs to the group of synthetic fluoroquinolone antibiotics with broad antimicrobial activity[1], and is structurally related to nalidixic acid. It is believed that the mode of action of this family of drugs is through binding DNA-gyrase enzyme[2]. It is also reported that there is a direct correlation of fluoroquinolone bonding with inhibition of DNA-gyrase enzyme activity and induction of DNA breakage. Because of this special mechanism of action, fluoroquinolones are considered to be the most effective gram-positive-gram-negative pathogens to combat infections caused by micro organisms that are resistant to other microbials, such as tetracycline. The drug is official in British Pharmacopoeia[3] and United States Pharmacopoeia[4] which describe a high performance liquid chromatographic (HPLC) method for its assay.

Assay of CPF in pharmaceuticals has previously been achieved by several analytical techniques such as HPLC[5-10], HPTLC[11], capillary electrophoresis[12,13], high performance capillary electrophoresis[14], fluorimetry[15] spectrofluorimetry[16], chemiluminometry[17], ISE-based potentiometry[18] and voltammetry[19,20]. However, many of these require expensive equipment and skilled operation. UV-spectrophotometry has also been used for the assay of CPF in single dosage forms[9,] and in two component mixture[21,22].

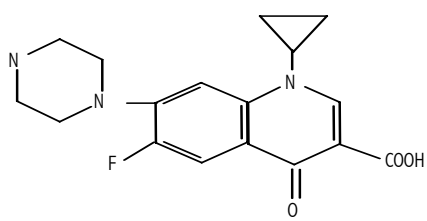

Fig 1. Structure of Ciprofloxacin.

Literature survey revealed that only two titrimetric methods have been proposed for the assay of CPF in dosage forms. The acid dye biphasic titration proposed by Zhang et al[23] is performed in aqueous-$CHCl_3$ medium whereas the non aqueous titrimetric procedure[24] of Kilic et al is applicable over 15-50 mg range. Numerous visible spectrophotometric methods based on redox[25], oxidative-coupling[26], binary complexation[27-28], ternary complexation[29], charge-transfer complexation[30,31] and ion-pair complexation[32-33] reactions are found in the literature for the assay of CPF in formulations. However, most of these methods suffer from such disadvantage as poor sensitivity[25-29], heating[30] or extraction step[32,33] as shown in Table 1.

In this communication, we demonstrate the use of titrimetric and spectrophotometric techniques for the assay of CPF using cerium (IV) sulphate.

Materials and Methods

Apparatus

All absorbance measurements were made with a Systronics Model 106 digital spectrophotometer provided with matched 1-cm quartz cells.

Reagents and Solutions

All chemicals used were of analytical reagent grade and distilled water was used to prepare all solutions.

Cerium (IV) Sulphate (0.025M): A 0.025 M cerium (IV) sulphate solution was prepared by dissolving about 10 g of the chemical (Loba-Chemie Indoaustranal Co., India) in 1.0 M sulphuric acid and diluting to 1 litre with the same acid and standardized with a solution of ammonium ferrous sulphate. The stock solution (0.025 M) was used for titrimetric work and diluted appropriately with 1M sulphuric acid to yield 250 & 500 μg ml^{-1} cerium (IV) sulphate solutions for use in spectrophotometric method B and method C, respectively.

Ferrous ammonium sulphate (0.025M): A 0.025 M FAS solution was prepared by dissolving 9.80 g of the chemical (Qualigens India Ltd., Mumbai, India) in acid and diluting to 1 litre with the water and standardized with pure potassium dichromate and the stock solution was used for titrimetric work

Methyl Orange (50 μg ml^{-1}): A 500 μg ml^{-1} solution was first prepared by dissolving 59 mg of dye (S. d. Fine Chem, Mumbai, India; dye content 85 %) in water and diluting to the mark in a 100 ml calibrated flask and filtered. This was diluted 10-fold to obtain a working concentration of 50 μg ml^{-1}.

Indigo carmine (200 μg ml^{-1}): A 1000 μg ml^{-1} solution was first prepared by dissolving 111 mg of dye (S. d. Fine Chem., Mumbai, India; dye content 90 %) in water and diluting to the mark in a 100 ml calibrated flask and filtered. This was appropriately diluted to get 200 μg ml^{-1} solution with water.

Sulphuric acid (5 M): A 272 ml of concentrated sulphuric acid (S. d. Fine Chem, Mumbai, India; Sp gr 1.84) was added to 728 ml water with cooling.

Ferroin indicator: A 0.07 g of iron (II) sulphate heptahydrate and 0.15 g of 1,10-phenanthroline hydrate were dissolved in 10 ml of distilled water.

Standard Ciprofloxain Solution

A stock standard solution containing 2 mg ml^{-1} CPF was prepared by dissolving 500 mg of pure sample (Torrent Pharmaceuticals, Ahmedabad, India) in water

Table 1. Comparision of the existing spectrophotometric methods with the proposed methods.

Sl No	Reagent/s used*	λ_{max}, nm	Linear range (μg ml^{-1}) ε (l mol^{-1} cm^{-1})	Remarks	Ref.
1	Cerium (IV) sulphate	345	12-120 ($5.1x 10^3$)	Involves extraction into $CHCl_3$	25
2	Iron (III) - MBTH	425	6-12	30 min contact time, less stable colour, expensive reagent	26
3	Iron (III) chloride	432	50-125	Less sensitive	27
4	Iron (III)	447	50-500	Uses flow injection automated assembly, less sensitive	28
5	Iron (III) nitrate	375	35-300	Less sensitive	29
6	TCBQ	376	0.9-25 ($1.27x10^4$)	Involves heating at 35ÚC for 30 min	30
7	p-benzoquinone	495		Involves rigid pH control	31
8	a)Bromocresol Purple	410	1.5-16.5 ($1.7x10^4$)	Involves rigid pH control and liquid - liquid extraction	32
	b) Bromophenol Blue	410	1.5-12.0 ($1.6x10^4$)		
9	Supracene Violet 3B	575	2.5-30 ($8.62x10^3$)	Involves rigid pH control and liquid - liquid extraction	33
10	a. Ce (IV) sulphate-MO	520	0.5-3.5 ($6.1 x 10^4$)	Non-stringent working conditions, no heating or no extraction step, highly sensitive	Present methods
	b. Ce (IV) sulphate - IC	610	1.0 -7.0 ($3.0 x 10^4$)		

* MBTH, 3-methyl-2-bezothioazolin-2-one-hydrazone; TCBQ, Tetrachlorobenzoquinone; MO, Methyl orange; IC, Indigo carmine.

and diluting to the mark in a 250 ml calibrated flask, and used in titrimetric work. This solution (2000 μg ml^{-1} CPF) was diluted stepwise with water to obtain working concentrations of 10 and 20 μg ml^{-1} for investigations by spectrophotometric method B and method C, respectively.

General Procedures

Titrimetry (Method A): A 10-ml aliquot of standard drug solution containing 2-12 mg of CPF was placed in a 100-ml titration flask and the solution was acidified by adding 5 ml of 5 M sulphuric acid. Then, 10 ml of 0.025 M cerium (IV) sulphate solution was added by means of a pipette, the contents mixed well and the flask set aside for 15 min. Finally, the unreacted oxidant was back titrated with 0.025 M FAS solution using one drop of ferroin indicator. Simultaneously, a blank titration was performed, and the amount of drug in the measured aliquot was calculated from the amount of cerium(IV) reacted.

Spectrophotometric method B (Methyl orange): Different aliquots (0.5-3.5 ml) of a standard 10 μg ml^{-1} CPF solution were transferred into a series of 10 ml calibrated flasks by means of a micro burette and the total volume was adjusted to 4.0 ml by adding water. To each flasks were added 1 ml each of 5 M sulphuric acid and 250 μg ml^{-1} cerium (IV) sulphate solutions, mixed well and the flasks were kept aside for 10 min with occasional swirling. Then, 1 ml of methyl orange solution was added and the volume was diluted to the mark with water and mixed. The absorbance of each solution was measured at 520 nm against a water blank after 5 min.

Spectrophotometric method C (Indigo carmine): Varying aliquots (0.5-3.5 ml) of a standard 20 μg ml^{-1} CPF solution were accurately measured into a series of 10 ml calibrated flasks and the volume was adjusted to 4.0 ml by adding requisite quantity of water. To each flask were added 1 ml of 5 M sulphuric acid followed by 1 ml of 500 μg ml^{-1} cerium (IV) sulphate solutions. The contents were mixed well and the flasks set-aside for 10 min. Then, 1 ml of indigo carmine solution was added, the volume was diluted to the mark with water, and mixed well. The absorbance of each solution was measured at 610 nm against a water blank after 5 min.

The concentration of the unknown was read from the calibration graph or computed from the respective regression equation.

Procedure for Formulations

Twenty tablets were weighed and ground into a fine powder. An amount of powder equivalent to 500 mg of CPF was accurately weighed into a 250 ml calibrated flask, 60 ml of water added and the mixture shaken for 20 min. Then, 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 was subjected to analysis by titrimetry. The tablet extract (2000 μg ml^{-1}) was suitably diluted to get 10 and 20 μg ml^{-1} CPF and analysed spectrophotometrically by taking a convenient aliquot.

Results and Discussion

The proposed methods are indirect and are based on the determination of the residual cerium (IV) sulphate after the reaction between CPF and the oxidant is ensured to be complete in acid medium. The amount of oxidant reacted corresponds to the amount of drug in all the methods.

Titrimetry: Direct titration of CPF with cerium (IV) sulphate was not successful. Hence, several experimental variables were optimized for the indirect determination. Reproducible and stoichiometric results were obtained when 0.2-2.0 M sulphuric acid concentration was maintained, and hence a 1.0 M acid concentration was used throughout (Fig.2). The

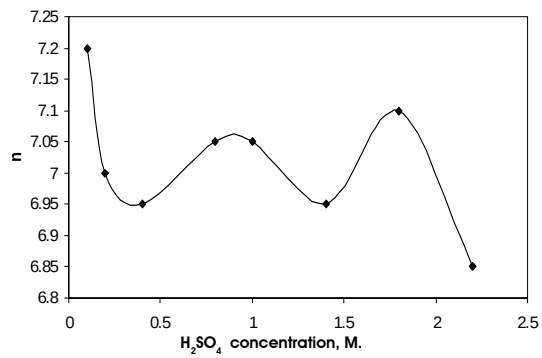

Fig 2. Effect of H_2SO_4 concentration.

reaction was found to be quantitative with a stoichiometry of 1:7 (CPF: oxidant) for the range studied (2-12 mg). The reaction was found to be complete in 15 minutes yielding a stoichiometry of 1:7. Contact times beyond 15 minutes and up to 40 minutes consumed a small amount of oxidant, but without yielding any significant stoichiometry (Fig.3). Hence it is necessary that the oxidation reaction be terminated after 15 minutes.

Since seven moles of oxidant are consumed by each mole of CPF, the reaction path way is unclear. However, additional study will be performed to reveal the reaction mechanism.

Spectrophotometric methods: The ability of cerium (IV) sulphate to cause oxidation of CPF and bleach the colour of methyl orange and indigo carmine dyes has been used for the indirect spectrophotometric

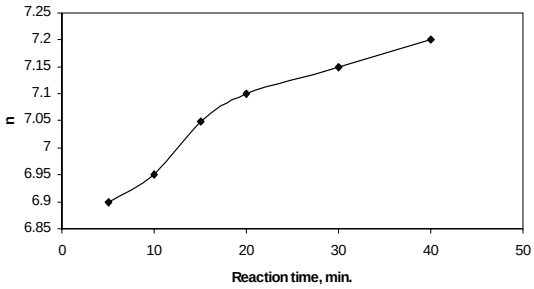

Fig 3. Effect of reaction time.

assay of CPF. In both methods, the drug was reacted with a measured excess of cerium (IV) sulphate in acid medium and the unreacted oxidant was determined by reacting with either methyl orange or indigo carmine followed by absorbance measurement at 520 or 610 nm. In either method, the absorbance increased linearly with increasing concentration.

CPF, when added in increasing amounts to a fixed amount of cerium (IV) sulphate, consumed the latter and there occurred a concomitant fall in its concentration. When fixed amount of either dye was added to decreasing amounts of oxidant, a concomitant increase in the concentration of dye resulted. This was observed as a proportional increase in absorbance at the respective λ_{max} with increasing concentration of CPF (Fig. 4 and 5).

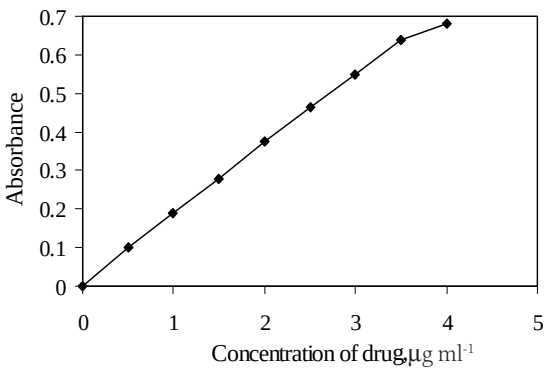

Fig 4. Beer's law curve for method B.

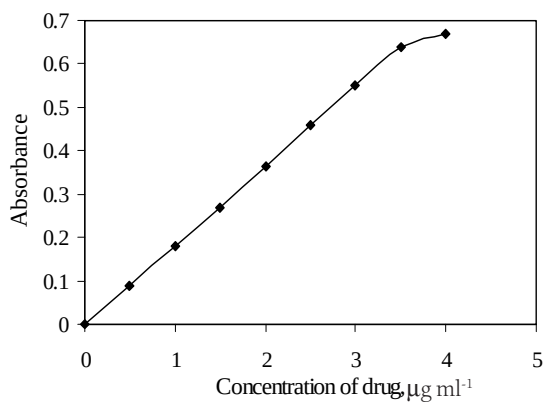

Fig 5. Beer's law curve for method C.

Preliminary experiments were performed to fix the maximum concentrations of the dyes that could be determined spectrophotometrically, and these were found to be 5 and 20 μg ml^{-1} for methyl orange and indigo carmine, respectively. A cerium (IV) sulphate concentration of 25.0 μg ml^{-1} was found to destroy the red colour due to 5 μg ml^{-1} methyl orange whereas in the case of 20 μg ml^{-1} indigo carmine, 50.0 μg ml^{-1} cerium (IV) sulphate was sufficient to bleach the blue colour in acid conditions. Hence, different amounts of CPF were reacted with 1.0 ml of 250 μg ml^{-1} oxidant in method B and 1.0 ml of 500 μg ml^{-1} oxidant in method C before determining the residual cerium (IV) sulphate as described under the respective procedures.

The reaction was carried out in sulphuric acid medium. One ml of 5 M acid was used in the assay procedures. For quantitative reaction between CPF and cerium (IV) sulphate a contact time of 10 min was found sufficient in both methods and constant absorbance readings were obtained when contact times were extended upto 30 min. The standing time of 5 min was necessary for the bleaching of dye colour by the residual oxidant. The measured colour was stable for hours in the presence of reaction product.

Analytical Parameters of the Spectrophotometric Methods

A linear relation was found between absorbance at λ_{max} and concentration of CPF in the ranges shown in Table 2. The calibration graphs are described by the equation:

$Y = a + b X$

(where Y = absorbance, a = intercept, b = slope and X = concentration in μg ml^{-1}) obtained by the method of least squares. The apparent molar absorptivity and Sandell sensitivity values together with the limits of detection and quantification compiled in Table 2 are indicative of the high sensitivity of the proposed methods.

Table 2. Analytical parameters for spectrophotometric methods.

Parameter	Method B	Method C
λ_{max}, nm	520	610
Beer's law limits, μg ml^{-1}	0.5-3.5	1.0-7.0
Molar absorptivity, l mol^{-1} cm^{-1}	6.1×10^4	3.0×10^4
Sandell sensitivity, μg cm^{-2}	0.005	0.011
Limit of detection, μg ml^{-1}	0.05	0.09
Limit of quantification, μg ml^{-1}	0.16	0.31
Regression equation, Y*		
Intercept (a)	8.6×10^{-3}	1.8×10^{-3}
Slope (b)	0.18	0.09
Correlation coefficient (r)	0.9971	0.9897

*Y= a + b X where Y is the absorbance, a intercept, b slope and X concentration in μg ml^{-1}.

Table 3. Evaluation of accuracy and precision.

Method*	CPF Taken	CPF Found	Range	Relative error, %	SD	RSD, %
Titrimetry (method A)	4	4.09	0.36	2.25	0.03	0.77
	8	7.90	0.30	1.13	0.08	1.02
	12	11.8	0.34	1.67	0.20	1.74
Spectrophotometric method B	1.0	0.98	0.17	2.00	0.02	2.33
	2.0	2.04	0.16	2.00	0.01	0.57
	3.0	2.98	0.19	0.66	0.04	1.46
Spectrophotometric method C	2.0	2.03	0.25	1.50	0.02	0.86
	5.0	4.96	0.29	0.80	0.10	2.12
	7.0	6.92	0.30	1.14	0.09	1.38

* In method A, CPF taken/found, range and SD are in mg while in methods B and C, the same are in $\mu g\ ml^{-1}$.
SD, standard deviation; RSD, relative standard deviation.

Method Validation

Accuracy and Precision

The accuracy and precision of the methods were evaluated by performing seven replicate analyses on pure drug solutions at three different amount/concentration levels (within the working ranges). The relative error (%), an indicator of accuracy was within 2.5% and intra day precision which is also called the repeatability expressed in relative standard deviation (RSD) (%) was also less than 2.5%, indicating the high accuracy and precision of the methods. The results of this study are compiled in Table 3. The reproducibility of the methods, also called the day-to-day precision, was assessed by performing replicate analyses on pure drug solutions at three levels over a period of five days preparing all solutions afresh each day. The day-to-day RSD values were less than 4% reflecting the usefulness of the methods in routine use.

The accuracy and precision of the methods were further assessed by performing recovery experiments. To a fixed amount of drug in the dosage form (pre-analyzed) pure drug was added at three different levels and the total was found by the proposed methods. Each test was performed in triplicate. The percent recoveries of the added pure CPF were in the range of 98.68-103.72 (Table 4) revealing good accuracies and non-interference from excipients and diluents such as talc, starch, gelatin, gum acacia, calcium carbonate, calcium gluconate, calcium dihydrogen orthophosphate, sodium alginate and magnesium stearate. This was further confirmed by the fact that no more than the stoichiometric amount of cerium (IV) was consumed when the tablet extract/injection solution was treated with cerium (IV) under the described experimental conditions.

Application

Commercial tablets and injections containing CPF were successfully analysed by the proposed methods. Co-formulated substances did not interfere. For the purpose of comparison, the same batch tablets and injections were analysed by an established method[9]. The results of the assay are presented in Table 5. As shown in Table 4, the results of analysis obtained by the proposed methods are in conformity with those obtained by the reference method[9]. The performance of the methods was further ascertained by applying Student's t-test for accuracy and F-test for precision. At the 95% confidence level, the calculated t- and F-values did not exceed the tabulated values (t = 2.77 and F =

Table 4. Results of analysis of dosage forms containing CPF.

Brand name[ψ] and dosage form	Label claim, mg/tablet or mg/ml	% found* ± SD			
		Reference method	Method A	Method B	Method C
Ciprolet[a] tablets	100	99.36 ± 0.74	100.36 ± 0.86, t = 1.77, F = 2.35	99.84 ± 1.26, t = 1.09,F = 2.90	101.72 ±1.55, t = 2.27,F = 4.38
Quintor[b] tablets	250	101.72 ±1.26	100.3 ± 1.82, t = 1.52,F = 2.08	99.3 ± 1.28, t = 2.18,F = 1.03	99.61 ± 1.13, t = 2.10,F = 0.80
Quintor[b] injections	2	100.28 ±0.91	98.67 ± 1.16, t = 0.79,F =1.62	99.97 ± 1.64, t =0.78, F = 3.25	101.34± 1.45, t = 1.50,F = 2.53

* Mean value of five determinations.
ψ Marketed by: a. Dr. Reddy's Laboratories.
b. Torrent pharmaceuticals.
Tabulated t-value at 95% confidence level is 2.77.
Tabulated F-value at 95% confidence level is 6.39.

Table 5. Results of recovery study by standard-addition method.

Formulation studied	Titrimetry (method A)				Spectrophotometry (Method B)				Spectrophotometry (Method C)			
	Amount of CPF in formulation, mg	Amount of pure CPF added, mg	Total found, mg	% recovery of pure CPF *	Amount of CPF in formulation, µg	Amount of pure CPF added, µg	Total, found µg	% recovery of pure CPF *	Amount of CPF in formulation, µg	Amount of pure CPF added, µg	Total found, µg	% recovery of pure CPF *
Ciprolet	4.02	2.0	6.05	101.3	10.03	5.0	14.96	98.50	20.3	10	30.45	101.5
tablet	4.02	4.0	7.97	98.68	10.03	10.0	19.79	97.60	20.3	20	40.07	98.84
(100 mg)	4.02	6.0	9.97	99.14	10.03	20.0	30.51	102.40	20.3	40	60.42	100.3
Quintor	3.95	2.0	5.91	98.15	9.87	5.0	15.02	103.1	20.27	10	30.74	104.72
Injections	3.95	4.0	8.01	101.4	9.87	10.0	19.92	100.5	20.27	20	40.20	99.66
(2 mg ml^{-1})	3.95	6.0	9.94	99.78	9.87	20.0	29.49	98.10	20.27	40	60.95	101.70

*Mean value of three determinations.

6.39) suggesting that the proposed methods are as accurate and precise as the established/reference method.

Conclusions

Three new methods using cerium (IV) sulphate as the oxidimetric reagent have been developed for the determination of ciprofloxacin based on oxidation. The titrimetric method, although has a narrow dynamic range of applicability, offers a better sensitivity compared to the methods reported previously. The present spectrophotometric methods using cerium (IV) sulphate have long dynamic linear ranges of response and better sensitivity in terms of molar absorptivities and limits of detection and are free from boiling step compared to many of the previously reported procedures. The methods involve the measurement of stable coloured species, have shorter contact times and are free from extraction step. The other advantages include the use of cerium (IV) which is highly stable in solution. These advantages coupled with fairly high accuracy and precision of the methods render them suitable for routine use. However, the reaction time in titrimetry is some what critical and should be strictly adhered to.

Acknowledgements

The authors gratefully acknowledge the receipt of pure ciprofloxacin as gift from Torrent Pharmaceuticals Ltd, Ahmedbad, India. The authors (PN, BCS&VRK) thank the authorities of the University of Mysore, Mysore, for research facilities. One of the author (VRK) is thankful to the Principle Secretary, Dept of Health and Family Welfare, Govt of Karnataka, Bangalore, for permission.

References

1. Goodman LS and Gillman AG (1996) *The Pharmacological Basis of Therapeutics,* 9th ed (McGraw-Hill, NewYork) pp 1065.
2. Wise R, Andrews JM and Edwards LJ (1983) *Antimicrob Agents Chemother* **23,** 559.
3. *The British Pharmacopoeia* (1999) Vol 2 (Her Majesty's Stationery Office, London) pp 1714.
4. *The United States Pharmacopoeia 24* (1999) (United States Pharmacopoeial Convention, 12601, Twinbrook Parkway, Rockville, MD 20852) pp 420.
5. Zatou A and Miltiadou N (2002) Sensitive LC-determination of ciprofloxacin in pharmaceutical preparations and biological fluids with fluorescence detection, *J Pharm Biomed Anal* **28,** 559-68.
6. Vega E. Dabbane V, Nassetta M and Sola N (1999) Validation of reversed phase LC method for quantitative analysis of intravenous admixtures of ciprofloxacin and metronidazole, *J Pharm Biomed Anal* **21,** 1003-9.
7. Lacriox PM, Curran NM and Sears RW (1996) High pressure liquid chromatographic methods for ciprofloxacin hydrochloride and related compounds in raw materials, *J Pharam Biomed Anal* **14,** 641-54.
8. Husain S, Khalid S, Nagaraju V and Nageshwara Rao R (1995) High performance liquid chromatographic separation and determination of small amounts of process impurities of ciprofloxacin in bulk drugs and formulations, *J Chromatogr A* **705**, 380-4.
9. Liu RL, Xu JR, Liu YG and Yao Z (1994) Studies on methods for determination of ciprofloxacin hydrochloride, *Yaowu Fenxi Zazhi* **14,** 45-6.
10. Morley JA and Elrod L (1993) Determination of fluoroquinolone antibacterials as N-acyl derivatives, *Chromatographia* **37,** 295-9.
11. Novakovic J, Nesmerak K, Nova H and Filka K (2001) An HPTLC method for the determination and the purity control of ciprofloxacin HCl in coated tablets, *J Pharm Biomed Anal* **25,** 957-64.
12. Wang PL, Feng YL and Chen LA (1997) Validation of capillary electrophoresis method for the determination of quinolone antibiotic and its related impurities, *Microchem J* **56,** 229-35.
13. Altria KD and Chanter YC (1993) Validation of a capillary

electrophoresis method for the determination of a quinolone antibiotic and its related impurities, *J Chromatogr A* **652,** 459-63.
14. Yin C and Wu YT (1997) Determination of four quinolones by high performance capillary electrophoresis, *Yaowu Fenxi Zazhi* **17,** 371-3.
15. Zhou CJ, Liu N and Guo YE (1998) Fluorimetric determination of the content of ciprofloxacin hydrochloride ointments, *Yaowu Fenxi Zazhi* **18,** 200-1.
16. Drakopoulos AI and Ionnou PC (1997) Spectrofluorimetric study of acid-base equilibria and complexation behaviour of the fluroquinone antibiotic ofloxacin, norfloxacin, ciprofloxacin and pefloxacin in aqueous solutions, *Anal Chim Acta* **354,** 197-204.
17. Liang YD, Li JZ and Zhang Z J (1997) Flow injection chemiluminescence determination of ciprofloxacin hydrochloride, *Fenxi Huaxe* **25,** 1307-10.
18. Avsec H and Gomiscek S (1992) Study of the prospects for a ciprofloxacin PVC-coated wire ion-selective electrode on quinolin-4-ones, *Anal Chim Acta* **268,** 307-9.
19. Di JW and Jin M (1995) Study on the determination and electrode mechanism of ciprofloxacin by LSP, *Fenxi Shiyanshi* **14,** 33-6.
20. Chen Y, Han FM and Yuan ZB (1995) Single sweep polarographic analysis of ciprofloxacin, *Fenxi Kexue Xuebao* **11,** 55-7.
21. Zhang LT, Yuan ZF, Chen RH, Zhou ZX, Zhou YM, Zhang AH, Liu WN and Whang ZH (1997) Determination of ciprofloxacin hydrochloride in tablets by differential spectrophotometry, *Yaowu Fenxi Zazhi* **17,** 33-4.
22. Bungalowala A, Jain DK and Trivedi P (1998) Simultaneous determination of ciprofloxacin and tinidazole in tablet dosage forms, *Indian Drugs* **35,** 348-51.
23. Zhang SF, Sun ZX and Sun XL (1996) Determination of ciprofloxacin lactate in injections by acid dye biphasic titration, *Yaowu Fenxi Zazhi* **16,** 402.
24. Kilic E, Koseoglu F and Akay M (1994) A Non-aqueous titrimetric assay of selected antibiotics using tetrabutylammonium hydroxide as titrant, *J Pharm Biomed Anal* **12,** 347-52.
25. Bharat PV, Rajani G and Vanita S (1997) An oxidative spectrophotometric method for the determination of ciprofloxacin in pharmaceutical preparations, *Indian Drugs* **34,** 497-500.
26. Ramana Rao G, Avadhanulu AB and Vasta DK (1990) Spectrophotometric determination of ciprofloxacin in its dosage forms, *Indian Drugs* **27,** 532-5.
27. Bhowal SK and Das TK (1990) Spectrophotometric determination of some recently introduced antibacterial drugs using ferric chloride, *Anal Lett* **24,** 25-37.
28. Sultan SM and Suliman F O (1992) Flow-injection spectrophotometric determination of the antibiotic ciprofloxacin in drug formulations, *Analyst (London)* **117,** 1523-6.
29. Chowdary KPR and Rama Prasad YV (1994) Spectrophotometric estimation of ciprofloxacin hydrochloride in dosage forms and in dissolution rate studies, *Indian Drugs* **43,** 277-9.
30. Xuan CS, Ren SC, Song JL and Wang ZY (1996) Spectrophotometric determination of ciprofloxacin using tetrachlorobenzoquinone, *Yaowu Fenxi Zazhi* **16,** 164-6.
31. Shanbag SG, Thampi PP and Thampi CS (1990) Simple spectrophotometric method for the estimation of ciprofloxacin hydrochloride and its dosage forms, *Indian Drugs* **28,** 279-80.
32. Tosunoglu S and Savci N (1993) Two spectrophotometric methods for the determination of ciprofloxacin in pharmaceuticals with use of bromocresol purple and bromophenol blue, *Acta Pharm Turc* **35,** 1-5.
33. Sastry CSP, Rama Rao K and Prasad DS (2003) Extractive spectrophotometric determination of some fluoroquinoline derivatives in pure and dosage forms, *Talanta* **42,** 311-6.