*Proceedings of the Pakistan Academy of Sciences:*
*A. Physical and Computational Sciences* 56 (1): 53–57 (2019)

ISSN: 2518-4245 (print), 2518-4253 (online)

Pakistan Academy of Sciences

Research Article

# Validation of A Novel Atomic Absorption Spectrophotometric Technique in Determination of Ciprofloxacin Using High Pressure Liquid Chromatography as a Reference Method

**Amina Habib[1*], Farooq Saleem[1], Saeed-Ul-Hassan[1], and Habib Amjad[2]**

[1]Faculty of Pharmacy University of Lahore, Lahore
[2]Institute of Chemistry, University of the Punjab, Lahore

**Abstract:** Same quantities of ciprofloxacin (0.2, 0.4, 0.6, 0.8 and 1.00 µg/liter) were determined by using atomic absorption Spectrophotometric (AAS) and high pressure liquid chromatography (HPLC) techniques. AAS technique was introduced as the test method where HPLC method was applied as the reference method as to validate the results of test method. In HPLC method reverse phase C18 column (RPC18 column) was used where mixture of acetonitrile: Phosphate buffer (35:65) was employed as mobile phase. In AAS method 284 nm wavelength revealed the maximum absorption and therefore further all the measurements were recorded using this wavelength. In AAS test technique, at first step ciprofloxacin was fused with sodium biphenyl. Then sodium fluoride (NaF) formed was reacted with FeCl3 to give iron fluoride (FeF3). The extra amount of FeCl3 was removed by reacting it with ammonium thiocyanate. Ferric thiocyanate (FeSCN) was selectively dissolved in Methyl Isobutyl Ketone (MIBK) and separated by filtration. NH4Cl and ferric thiocyanate were held back. Amount of Fluorine (F) was determined in terms of Fe and using this amount, quantity of ciprofloxacin was calculated.

**Keywords:** Atomic Absorption Spectrophotometry (AAS), High Pressure Liquid Chromatography (HPLC), Methyl Isobutyl Ketone (MIBK), Ferric Fluoride ($FeF_3$), Ammonium Chloride ($NH_4Cl$), Sodium Fluoride (NaF), Ciprofloxacin (CIP), Reverse Phase Column 18 ($RPC_{18}$).

## 1. INTRODUCTION

High Pressure Liquid Chromatography (HPLC) is a cumbersome, laborious and expensive technique to take readings in an organic analysis. However, analysis by atomic absorption Spectrophotometric (AAS) technique is not only novel, time saving and but also easier to carry out. AAS technique is generally used for inorganic analysis but in this method, it has been validated for organic analysis using antibiotic ciprofloxacin. The rate of chicken growth has increased throughout the world. So the rate of infections and diseases also has been increased. A very large amount of flouroquinolones antibiotics are given to the poultry industry to prevent these infectious diseases. Low dose, long-term treatment of poultry with flouroquinolones enhances the growth. But in this way antibiotics are deposited and accumulated within the tissue [1]. It goes to the poultry products and edibles. For example, chicken muscle is used in patties and fruit-cake. In this way the deposited amounts of antibiotics are liable to be found in such products. As these products are used as food, therefore, it becomes vital to analyze the edibles for the contaminated antibiotics and compare them with international norms for their certification. The validation of AAS technique confirmed that the analysis can be done at the accelerated rates [2-5]. Oxytetracycline, sulfadimidine and oxolinic acid residues were determined from animal drug residues in meat and fish samples of meat and eggs. Prolonged oral use of different antibiotics like bacitracin and chlortetracycline residues have effects on slaughtered animals and rat tissues reported by Vavrova, et al., in 1971. [6]. A study

Received: April 2018; Received: March 2019
* Corresponding Author: Amina Habib <sadiahabibamjad@gmail.com>

conducted on the heat treatment effect by Roan et al., in 1981, revealed the effect of heat on the antibiotics residues in animal products. The antibiotics that were more resistant to boiling were Liver antibiotics [7].

Sulfonamides and tetracyclines cause drug contaminations on meat of animals that were treated with pharmaceuticals. The residues of antibiotics were identified on the tissues of the slaughtered animals by Tropilo et al [8]. The residues of tetracycline in animal liver were extracted by using $C_{18}$ cartridge packing in 1985 by Oka et al., [9]. In 1989, by the use of liquid and solid phase extraction columns, Rogstad et al., withdrawing oxolinic acid and flumiquine from fish tissues [10]. Prior to the development of effective modern antibacterial, serious system bacterial infections were feared as is AIDS today.

About 60 % of poultry product samples were contaminated of national origin with *Salmonella* and was observed by Antunes et al., while enrofloxacin and nalidixic acid resistance was determined in 50% of the isolates [11].

Identification and Quantification of flouroquinolones in eggs was done by Martin D.R et al., using extended multi-residue methodology [12]. The poultry industry uses fluoroquinolones like ciprofloxacin, enrofloxacin. These antibiotics are effective against Gram positive as well as Gram negative infections by the inhibition of DNA gyrase formation [13].

AAS technique was used to determine different antibiotics e.g. ciprofloxacin, by forming ion-association complexes and got precipitated at its optimum conditions and the excess unreacted cobalt complex was used for determination [14]. Bismuth tetraiodate complex was used for the determination and identification of pure antibiotics like ciprofloxacin, norfloxacin and levofloxacin. In AAS technique, the basic principle was ion-association complex formation. Ciprofloxacin, norfloxacin, ofloxacin and enrofloxacin were identified by AAS, colorimetric and conductometric methods. Working principle of methods was reaction of ammonium reineckate with these drugs to form stable precipitate of ion-pair complexes.

## 2. MATERIALS AND METHODS

### 2.1. Chemicals

Ciprofloxacin, Acetonitrile, Acetic Acid, Methanol, HCl, n-Hexane, n-Butanol, Activated Charcoal, NaOH, $FeCl_3$, Oxalic Acid, Sodium dihydrogen phosphate, Disodium hydrogen phosphate, Sodium biphenyl, Ammonium thiocyanate, SCX-Bond elute Cartridges, PSA-Bond elute Cartridges, Sodium Dodecyl Sulphate, anhydrous sodium sulphate, MIBK, (Merck).

### 2.2 Instruments & Apparatus

High Pressure Liquid Chromatography (Shimadzu), Atomic Absorption Spectrophotometer, Electronic oven(DHAUS), Analytical balance, p H meter (ADWA, AD1030), ultrasonic cleaner, Filtration assembly, filter paper whatmann no 42, 0.45µm nylon filter, glass filter, suction pump, column ODS C18, Cartridges (SCX and PSA), Microliter syringe 20µl, stirrer with hot plate.

### 2.3 Glassware & other Equipment

Cylinders, Beakers (10, 50, 100, 250, 500, 1000ml), Conical flasks, Glass funnels, solution vials (2ml and 6ml), Volumetric flasks (25, 50, 100,250, 500, 1000 ml), micropipettes and test tubes. Ciprofloxacin was determined indirectly by atomic absorption Spectrophotometric technique by reacting it with sodium biphenyl. Ciprofloxacin was decomposed by reaction with sodium biphenyl. Fluorine from the ciprofloxacin was converted to NaF. Then aqueous solution of NaF was maintained at ph of 3.0 with 0.2 N HCl. To 5 ml of this solution, 1 ml of $FeCl_3$ solution containing 10 mg per ml iron was first added. Then secondly, 1 ml 2.5 % ammonium thiocyanate solution was added. The excess iron was extracted as ferric thiocyanate by selectively dissolving it in methyl isobutyl ketone (MIBK) and filtering. Then iron in thiocyanate was determined by atomic absorption Spectrophotometric technique. The remaining iron had reacted with fluorine to form $FeF_3$. The amount of iron in $FeF_3$ was calculated by subtracting amount of iron in ferric thiocyanate obtained by atomic absorption spectrophotometer, from the given amount of iron, 50 mg in 5 ml iron chloride solution originally taken. Three atomic moles of fluorine are

present per atomic mole of iron. Hence, Fluorine is estimated. Ciprofloxacin is equimolar to atomic moles of fluorine. Hence amount of ciprofloxacin is given as, atomic moles of fluorine multiplied by molar mass of ciprofloxacin. Atomic moles of fluorine × 331.35 gm per given sample [15-16].

### 2.4 Optimization of CIP Sodium Biphenyl Ratio

Different amounts of sodium biphenyl ranging from 1 milli mole to 10 milli moles were fused separately with a fixed amount of CIP; 10 milli moles. Previously settled optimum concentration of $FeCl_3$ (0.08 mg/ml) was used to form CIP-$FeCl_3$ metal complex at pH 4.0. The same was repeated with the standards of CIP formed in 0.02 N HCl. Absorbances of samples and standards were taken at 284 nm. The concentration of sample befitting minimum absorbance was 5 milli moles of sodium biphenyl, showing maximumly used up CIP.

## 3. RESULTS & DISCUSSION

Sodium biphenyl reacts with ciprofloxacin completely in quite normal condition. One mole of Ciprofloxacin completely reacts with half mole of sodium biphenyl giving sodium fluoride. The number of atomic moles of fluorine is equivalent to the number of moles of ciprofloxacin. Sodium fluoride, thus obtained is reacted with excessive amount of $FeCl_3$ of analytical grade. $FeCl_3$ in excess to the reaction is further reacted with ammonium thiocyanate. The ferric thiocyanate thus formed is selectively dissolved in Methyl Isobutyl Ketone (MIBK) and the iron is quantified on Atomic Absorption Spectrophotometer (AAS). Using this amount of iron, iron in $FeF_3$ is quantified and fluorine from ciprofloxacin calculated and ciprofloxacin

**Table 1.** Data of Reference Method

| HPLC "Reference Method" ( Conc. µg/liter ) | AAS "Test Method" (Conc. µg/liter) |
|---|---|
| 0.200 | 0.198 |
| 0.400 | 0.412 |
| 0.600 | 0.599 |
| 0.800 | 0.804 |
| 1.150 | 1.045 |

**Table 2.** Data of Reference Method

| Xi | $Xi^2$ |
|---|---|
| 0.200 | 0.0400 |
| 0.400 | 0.1600 |
| 0.600 | 0.3600 |
| 0.800 | 0.6400 |
| 1.150 | 1.3225 |
| Σ=3.15 | Σ=2.5225 |

**Table 3.** Data of Test Method

| Xi | $Xi^2$ |
|---|---|
| 0.198 | 0.003904 |
| 0.412 | 0.169744 |
| 0.599 | 0.358801 |
| 0.804 | 0.646416 |
| 1.045 | 0.311025 |
| Σ=9.9729 | Σ=2.4899 |

**Table 4.** Data of Test Method

| HPLC method Conc. Of CIP µg/ Lit | New AAS method Conc. Of CIP µg/ Lit | Di | Di-$\bar{D}$ | (Di-$\bar{D}$)2 |
|---|---|---|---|---|
| 0.200 | 0.198 | 0.002 | -0.0164 | 0.00026896 |
| 0.400 | 0.412 | -0.012 | -0.0304 | 0.00092416 |
| 0.600 | 0.599 | 0.001 | -0.0174 | 0.00030276 |
| 0.800 | 0.804 | -0.004 | -0.0224 | 0.00050176 |
| 1.150 | 1.045 | 0.105 | 0.0866 | 0.00665856 |

also quantified by the use of amount of fluorine. Optimization was used to rationalize the reaction between sodium biphenyl and ciprofloxacin. The optimum amounts are equal to the experimental ratio. International criteria for validation of an analytical project are used as instruments of validation. These are "F "test and "t" test.

## 4. CONCLUSION

The table value at 95% confidence level and 4 degrees of freedom (n-1 or 5-1) is 2.776. As the calculated "t" "0.8865" was smaller than that tabulated "2.776" at 95% confidence level, hence both the methods have statistically no difference. The new method using AAS for flouroquinolones is validated according to international norms.

## 5. ACKNOWLEDGEMENTS

I feel honor to acknowledge the most inspiring personality my supervisor Sir Farooq Saleem PhD (PU) for his guidelines and encouragement. I feel very pleasure and honour to acknowledge Dr. Syed Saeed-ul-Hassan PhD (PU), Dean of Pharmacy Department, University of Lahore, for sharing his valuable guidelines. I feel very lucky to have my father Dr. Habib Amjad PhD (PU) for supporting, guiding and motivating me in every step of my study and life. I m heartily thankful to Sir Umar (Pharmacist Analyst) at High Noon Laboratories for providing me the antibiotic standards.

## 6. REFERENCES

1. Rose, M.D., J. Bygrave, & G.W.F. Stubbing. Extension of multi-residue methodology to include the determination of quinolones in food*, Analyst,* 12: 2789-2796 (1998).
2. Joint WHO/FAO expert consultation on Diet, nutrition and the prevention of chronic diseases(2002). *WHO Technical Report Series 2003* (Tenchinal report series .916) Geneva, Switzerland WHO Publishers.
3. Lewicki, J. TYLOSIN: A review of pharmacokinetics, residues in food animals and analytical methods, retreived from: http://www.fao.org/tempref/AG/agn/food/tylosin_2006.pdf on October 4, 2019.
4. Joint Consultation WHO/FAO Expert on Diet, nutrition and the prevention of chronic diseases(2003). *World Health Organ Techincal report series 2004* (Report series No. 916:i-viii) Geneva, Switzerland WHO Publishers.
5. Hellyer, M., V. Piatnytskyi, & S. Nerpii. *Encyclopedia of exporting to the eu under the DCFTA*. CTA Economic Export Analyst Ltd, Harrow, United Kingdom (2015).
6. Vavrova, M, & J. Smejkal, Effect of giving Zn-bacitracin and chlortetracycline in feeds for long periods on the formation of residues of those antibiotics in the tissues of some fattening animals and rats. Preliminary communication*. Biol. Chem. Vyz. Zvirat, 7*: 3-13 (1971).
7. Siemann, M., L.I, Andersson, & K. Mosbach, Separation and detection of macrolide antibiotics by HPLC using macrolide-imprinted synthetic polymers as stationary phases*. The Journal of antibiotics, 50*(1): 89-91 (1997).
8. Tropiło, J., M. Szulc, & K. Leszczyńska, Residues of antibiotics and other inhibitory substances in the tissues of abattoir animals*. Annals of the National Institute of Hygiene, 36*(3): 207(1995).
9. Hisao, O., H. Matsumoto., K. Uno., K.I, Harada., S. Kadowaki, & M. Suzuki, Improvement of chemical analysis of antibiotics: VIII. Application of prepacked C18 cartridge for the analysis of tetracycline residues in animal liver. *Journal of Chromatography A, 325*: 265-274 (1985).
10. Rogstad, A., V. Hormazabal, & M. Yndestad,

Extraction and high performance liquid chromatographic determination of enrofloxacin in fish serum and tissues. *Journal of liquid chromatography, 14*(3): 521-531(1991).

11. Bette. H, Drugs online face scrutiny, *Chemical & Engineering News*, 79(40): 1-51(2001).
12. Chang, C.S., W.H. Wang, & C.E. Tsai, Simultaneous determination of 18 quinolone residues in marine and livestock products by high performance liquid chromatography. *Journal of Food and Drug Analysis,* 18(2):87-97(2010).
13. Amjad, H., M. Naeem., J. Iqbal, & K. Khan, Estimation of selected residual antibiotics in poultry products available in local markets during summer*, Journal Of The Chemical Society Of Pakistan, 27*(6): 637-642 (2005).
14. Korany, M.A., H.Mahgoub., R.S. Haggag., MA.A. Ragab, & O.A, Elmallah, Chemometrics-assisted spectrophotometric green method for correcting interferences in biowaiver studies: Application to assay and dissolution profiling study of donepezil hydrochloride tablets, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy.* 199: 328-339 (2018).
15. Christian, G.D. *Data handling in: Analytical Chemistry.* John Wiley and Sons Inc. 5th edition, New York (1994).
16. Fiorino, J.A., J.W. Jones, G.C. Stephen, Sequential determination of arsenic, selenium, antimony, and tellurium in foods via rapid hydride evolution and atomic absorption spectrometry*, Analytical Chemistry,* 48(1): 120-125(1976).