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Quality assessment of various brands of Levofloxacin tablets available in Uyo Metropolis

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ABSTRACT

Levofloxacin is a second-generation fluoroquinolone antibacterial agent used for the treatment of a variety of bacterial infections, especially urinary tract infections, and prostitis. Ten brands of levofloxacin were sourced from various pharmacies in Uyo metropolis and subjected to qualitative and quantitative assay using standard physical tests and spectrophotometric method respectively. The standard physical tests done were weight uniformity, hardness, friability, disintegration and dissolution rate. Quantitative assay was by a spectrophotometric measurement of the ion-pair complex of the drug with the sulphonpthalein dye, bromothymol blue (BTB), using chloroform as the solvent. All the brands met the standard for weight uniformity test as they had percentage deviation of less than 5%. All the brands failed the hardness test as they had crushing strength of more than 10Kg/cm^2. All brands met the standard for friability test and disintegration test as they had percentage weight losses of less than 1% (w/w) and they all disintegrated in less than 15 minutes. Two brands did not meet the requirement for dissolution rate as they had less than 80% of the drug dissolving in 30 minutes. The spectrophotometric measurement was done at 410mn. The calibration curve for reference levofloxacin was linear over a concentration range of 2.5-40µg/mL and the recovery of the drug ranged between 99.04-100.09%. The ten brands were within the limits specified in the official compendia. The results obtained from these tests gave important information on the standard and batch-to-batch consistency of the evaluated brands. The spectrophotometric assay method is simple and precise for the analysis of levofloxacin in bulk and pharmaceutical dosage forms.

Keywords: Levofloxacin, Quality Assay, sulphonpthalein dye, bromothymol blue.

INTRODUCTION

Levofloxacin, (-)-(S)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1- piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid hemihydrate, the L-isomer of ofloxacin, is a broad-spectrum second generation fluoroquinolone antibacterial agent which acts by inhibiting the activities of bacterial DNA gyrase and topoisomerase IV, two bacterial type II topoisomerases that are key to bacteria regeneration and multiplication (Drlica and Xhao, 1997). It is greatly effective against both gram-negative and gram-positive bacteria that are resistant to other antibacterials (El-Brashy *et al.*, 2004). It is in the World Health Organisation's 19th Essential Drugs List (WHO, 2015), which potentially makes it a widely used drug. It is available orally, intravenously and as component of eye drops. Reports abound of fake, substandard or adulterated products in the market leading to undesired treatment outcomes (Cockburn *et al*., 2005; Genazzani and Pattarino, 2008; Igboasoiyi *et al*., 2012) making routine quality assessment of drug products in registered pharmacies a desirable and compelling activity. This research work was aimed at assessing if the brands of levofloxacin available in Uyo metropolis are of acceptable quality, using simple and cost-effective unsophisticated assay procedure to encourage its regular post-market surveillance. Various analytical methods have been reported for the determination of levofloxacin in dosage forms such as high performance liquid chromatography (HPLC) (Joshi, 2002), conductimetry (Altiokka *et al.*, 2002), high performance thin layer chromatography (HPTLC) (Mayyenathan *et al.*, 2003), atomic absorption spectrometry (Ragab and Amin, 2004), spectrophotometry (El-Brashy *et al.*, 2005), spectrofluorimetry (Salem *et al.*, 2007) and capillary electrophoresis (CE) (Elbashir, 2008), among others. Chromatographic methods have been extensively used and recommended. However, these methods generally require complex and expensive equipment, provision for use and disposal of solvents, labour-intensive sample preparation procedures and personal skills in chromatographic techniques. Few ultraviolet spectrophotometric methods have been reported for the determination of levofloxacin in dosage forms. The most widely used technique has been visible spectrophotometry and methods based in diverse reaction chemistries such as oxidative coupling reaction using Cerium(IV) with 3-methyl-2-benzo thiazolinehydrazone hydrochloride (MBTH) (Sastry *et al.*, 1995), ion-pair complexation with acid-dye such as tropaeolin OO and supracene violet 3B (Sastry *et al.*, 1995),

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or with bromophenol blue (BPB), and bromocresol purple (BCP) (Issa, *et al.,* 1997), charge transfer complexation with π-acceptors such as chloranilic acid, nitrophenol and tetracyanoquinodimethane (Mostafa *et al*., 2002), also complexation with tris(o-phenanthroline) iron (II) and tris(bipyridyl) iron (II), (Nagaralli *et al.,* 2002). Limitations of some of the above visible spectrophotometric methods include use of expensive reagents, use of heating step, poor sensitivity, liquid-liquid extraction step and close pH control. The present study describes direct, simpler, more sensitive and extraction-free technique for the determination of levofloxacin. The method employed the use of bromothymol blue (BTB) as a chromogenic agent and the resulting ion-pairs were measured directly in chloroform. The brands were assayed using the direct relationship between the intensity of the yellow-coloured complex formed, by the reaction of levofloxacin with bromothymol blue, and concentration of levofloxacin. Also described in this research are some official and non-official physical tests carried out on the selected commercial brands of levofloxacin. The official tests carried out include weight uniformity test, dissolution rate test and disintegration test, while the non-official tests carried out include friability and hardness tests. These tests were performed to evaluate the *in vitro* tablet characteristics (Ofoefule, 2002) of the selected brands.

MATERIALS AND METHODS

Materials: Ten different brands of levofloxacin tablets within their shelf-lives were purchased from community pharmacies in Uyo and coded A to J. The reagents used were of analytical grade and used as purchased.

Extraction of pure levofloxacin

Five tablets from the innovator brand of Levofloxacin (TAVANIC) we're powdered in a mortar. 100 mL of chloroform was added and allowed to stand for 15-20 minutes to allow for extraction of levofloxacin into the liquid phase. The mixture was filtered and the filtrate was placed in a petri dish to allow for evaporation of dichloromethane, leaving a dried levofloxacin powder which appeared as crystalline powder. The crystalline powder was subjected to melting point determination and ultraviolet spectrophotometry.

Weight Uniformity

Twenty tablets of each brand of levofloxacin were randomly selected and weighed individually on a Shimadzu, Japan analytical balance and the average weight calculated. The standard deviation and percentage deviation of each brand were calculated (table 1).

Friability test

Five tablets from each brand of levofloxacin were dedusted and weighed together (W_0). They were then placed in a 12-inch high drum Veego friability test apparatus and rotated at 25rpm for 4 minutes. The tablets were removed from the chamber, dedusted and reweighed (W).

The percentage weight loss was calculated with the formula: % weight loss = $\frac{W0 - W}{W0} \times 100$ (table 2).

Hardness test

Five tablets from each brand of levofloxacin were randomly selected and subjected to crushing force using the Monsanto hardness tester and the pressure at which the tablet crushed was recorded. The average crushing pressure required for each brand was determined and the results obtained are shown in table 3.

Disintegration test

Five tablets from each brand of levofloxacin was used for the test. 900 mL of distilled water was used as the disintegration medium with a bath temperature of $37^o \pm 0.5^oC$. The time taken for each of the tablets to disintegrate completely was recorded and the average disintegration time for each brand determined. The results are shown in table 4.

Preparation of Calibration Curve of Levofloxacin for Dissolution Test

Aliquots of 0.5, 1.0, 1.5, 2.0, and 2.5 mL of levofloxacin standard solution in 0.1N HCl (1mg/mL) were measured accurately and transferred into a series of 10 mL volumetric flask. These were diluted to the mark with 0.1N HCl and mixed well. The absorbance of each solution was measured using UV-VIS spectrophotometer at 293nm against the reagent blank. A standard graph was prepared by plotting the increasing absorbance values versus the concentration of levofloxacin (figure 1)

Dissolution test

This test was carried out using a digital tablet dissolution test apparatus. 0.1N HCl (900 mL) was used as the dissolution medium with bath temperature of $37^o \pm 0.5^oC$. For each brand of levofloxacin, the test was carried out in replicates. The duration of the test was 30 minutes and 10.0 mL dissolution samples were withdrawn at 5, 10, 15, 20, and 30 minutes and replaced with equal volume to maintain sink condition. The withdrawn samples were filtered using Whatman filter paper and assayed by UV-VIS spectrophotometer at 293 nm against the reagent

blank. The concentration of each sample was determined by extrapolation from a calibration curve of Levofloxacin in 0.1N HCl. The dissolution profiles of the various brands of Levofloxacin was then constructed (figure 2).

The difference factor (f1) and the similarity factor (f2).

The difference factor (f1) and the similarity factor (f2) were calculated to compare the dissolution profiles of the various brands with the innovator product (table 5)

Preparation of Standard solution (100 µg/L) for ion-pair Complexation Assay

10 mg of pure levofloxacin was weighed with an electronic balance (Shimadzu, Japan) and dissolved in 100 mL of chloroform. Determination of absorption spectrum of Levofloxacin-Bromothymol complex. 2.0 mL of levofloxacin standard solution was measured and transferred into a 10.0 mL measuring cylinder. 1.0 mL of 0.03% bromothymol blue in chloroform was added. The solution was mixed thoroughly and then scanned in the range of 360-480 nm against a reagent blank of 1.0 mL of 0.03 bromothymol blue in chloroform, made up to 10.0 mL with chloroform (figure 3).

Preparation of Calibration Curve of Levofloxacin for Ion-Pair Complexation

Aliquots of 0.25, 0.50, 1.00, 2.00, and 4.00 mL levofloxacin standard solution in chloroform (100.0 µg/mL) were measured accurately and transferred into a series of 5.0 mL volumetric flasks. 1.0mL of 0.03% bromothymol blue in chloroform was added to each flask, diluted to the mark with chloroform and mixed well. The absorbance of each resulting yellow chromogen was measured at 410 nm against the reagent blank. A standard graph was prepared by plotting the increasing absorbance values versus the concentration of levofloxacin (figure 4)

Preparation of Drug Solution

Each of the 10 brands of levofloxacin commercially obtained was prepared separately. For each brand, five tablets were accurately weighed and powdered. A portion equivalent to 10.0mg of levofloxacin was accurately weighed and transferred into a 100 mL volumetric flask, 10.0mL of chloroform was added to the flask and the content was shaken thoroughly for 15-20 minutes to extract the drug into the liquid phase, the volume was finally diluted to the mark with dichloromethane, mixed well and filtered using a Whatman filter paper.

Determination of Beer Lambert's Plot for Levofloxacin tablets.

Different concentrations were prepared for each brand of levofloxacin tablets as described for pure levofloxacin. The absorbance of each was measured at 410nm against the reagent blank. Their concentrations were calculated and also extrapolated from the calibration curve of levofloxacin using the formula:

Concentration = absorbance/slope

Their percentage recoveries were also calculated thus: $\frac{C_1}{C} \times 100$

Where C_1= extrapolated concentration

C= calculated concentration

RESULTS AND DISCUSSION

Table 1: Weight uniformity test results

Sample	A	B	C	D	E	F	G	H	I	J
Mean weight (mg)	822.3	616.3	607.3	596.1	680.0	921.0	749.3	599.0	634.2	677.8
SD (n=10)	±13.38	±5.02	±10.74	±5.43	±11.50	±4.12	±8.95	±5.81	±4.87	±5.50
% Deviation	1.63	0.82	1.77	0.91	1.69	0.45	1.19	0.97	0.77	0.81

Permissible percentage deviation is ≤ 5%

Table 2: Friability test results

Sample	A	B	C	D	E	F	G	H	I	J
W_0(g)	4.084	3.074	3.054	2.974	3.402	4.594	3.767	2.986	3.181	3.409
W(g)	4.075	3.066	3.041	2.965	3.394	4.586	3.758	2.976	3.174	3.404
W_0-W	0.009	0.005	0.004	0.009	0.008	0.008	0.009	0.010	0.007	0.005
% Weight loss	0.220	0.163	0.313	0.303	0.235	0.174	0.239	0.335	0.220	0.149

Permissible percentage weight loss is ≤ 1%

Table 3: Hardness test results

Sample	A	B	C	D	E	F	G	H	I	J
Average crushing strength (Kg/cm^2	14.6	14.8	14.5	11.9	14.5	14.1	14.2	10.9	14.2	14.3
±SD (n=10)	0.23	0.23	0.17	0.87	0.18	0.18	0.16	0.40	0.26	0.16

Permissible crushing strength is 4-10 Kg/cm^2

Table 4: Disintegration test results

Sample	A	B	C	D	E	F	G	H	I	J
Mean disintegration time (seconds)	217.0	508.0	484.4	306.4	473.2	528.4	612.2	195.0	747.2	406.8
±SD (n=10)	12.33	44.90	13.22	34.44	26.42	40.18	18.84	23.58	41.99	40.25
% Deviation	5.68	8.87	2.73	11.24	5.58	7.60	3.08	12.09	5.62	9.89

Permissible disintegration time is ≤ 15 minutes (900 seconds)

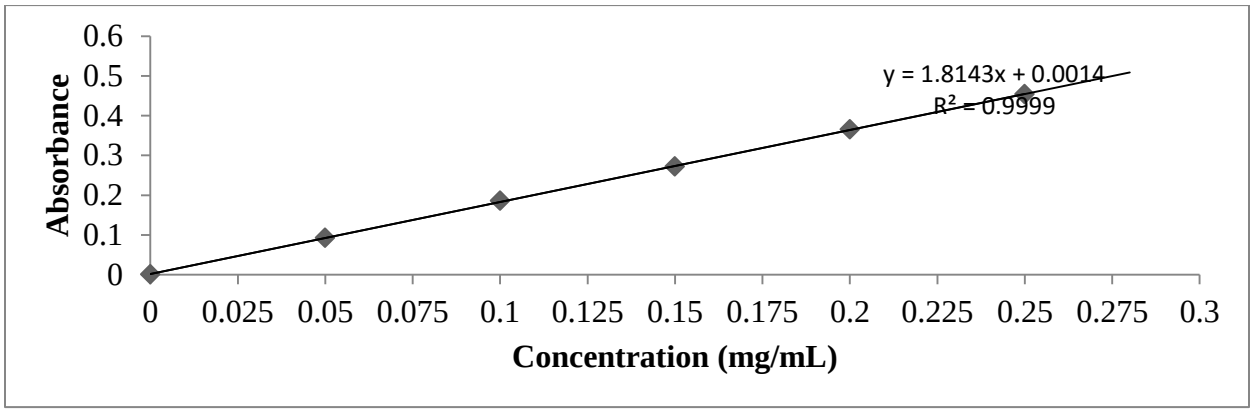

Figure 1: Calibration curve of levofloxacin in 0.1N HCl for dissolution test

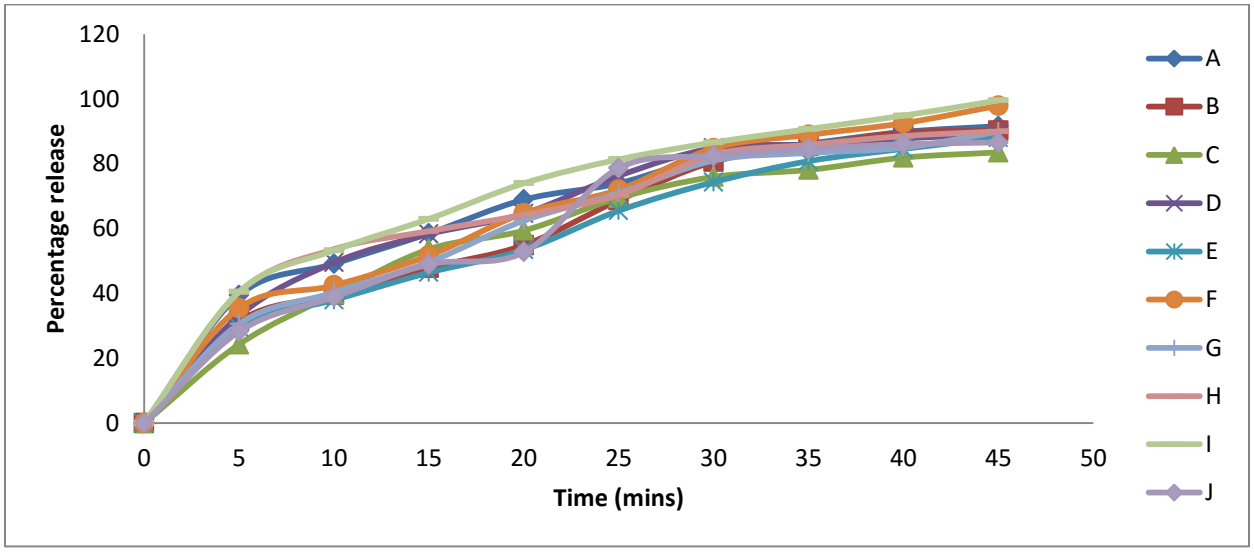

Figure 2: Dissolution profiles of different brands of levofloxacin tablets in 0.1N HCl

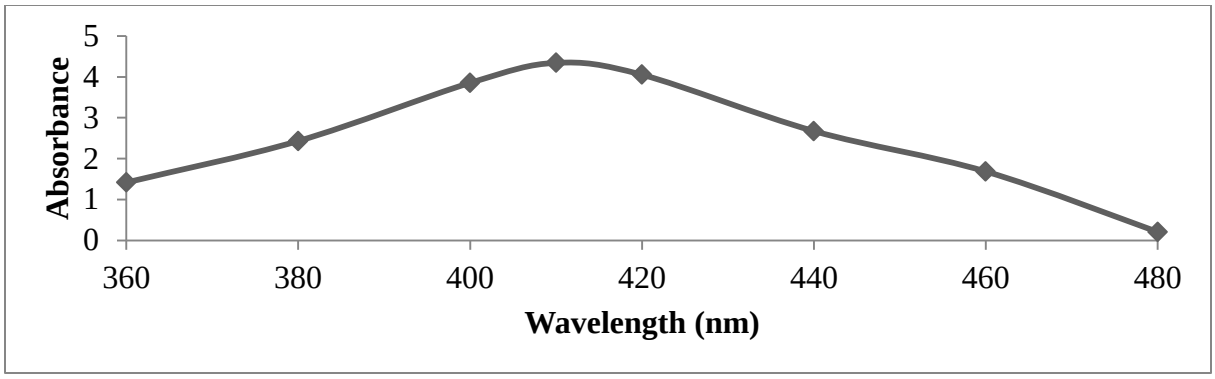

Figure 3: Ultra Violet visible spectrum of levofloxacin-bromothymol complex in chloroform

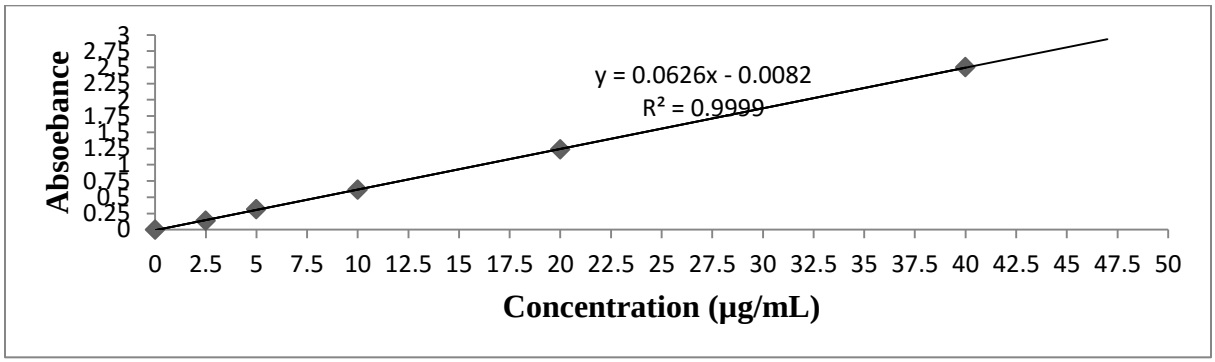

Figure 4: Calibration curve of levofloxacin-bromothymol complex for spectrophotometric assay

Table 5: f1 and f2 values of the various brands of levofloxacin tablets compared with the innovator brand I.

Sample	A	B	C	D	E	F	G	H	J
f1	6.37	13.95	17.18	7.92	17.80	7.92	13.26	7.13	14.09
f2	66.82	49.89	46.92	66.58	45.61	59.96	53.67	61.65	48.99

Table 6: Percentage recovery of different brands of levofloxacin

Sample	A	B	C	D	E	F	G	H	I	J
Percentage content %	99.04	99.77	99.55	99.55	99.50	99.24	99.89	99.88	100.09	99.15
±SD (n=5)	2.53	1.62	0.69	0.63	1.21	1.95	0.86	1.22	1.12	1.04

DISCUSSION

Uniformity of weight is an in-process test parameter which ensures consistency of dosage units. Weight of tablets is measured to ensure that a tablet contains the proper amount of drug. The uniformity of weight determination for the ten brands of levofloxacin gave values that complied with the USP specifications for film-coated tablets (Table 1) with a deviation less than 5% from the mean value (Maximum deviation value = 1.77%). The strict adherence to good manufacturing practice (GMP) during the granulation and compression stages ensures tablet weight uniformity. Tablets require a certain amount of strength, or hardness to withstand mechanical shocks of handling in manufacture, packaging and shipping. Friability is a tablet property that is related to the hardness of the tablet, it is the tendency of tablets to powder, chip or fragment. All the brands passed the friability test having weight loss of less than 1% (w/w). All the brands failed the hardness test as they did not meet the requirements for crushing strength (i.e. 4-10Kg/cm^2).

The crushing strength needed to crush the tablets were higher than the prescribed upper limit. The harder a tablet, the less friable it is and therefore will take more time to disintegrate. If the hardness of the tablets increases disintegration time beyond acceptable limits, it may likely affect treatment outcome. Disintegration test is essential for tablets intended for administration by mouth. Disintegration which is the breakdown of the tablets into smaller portions is an important step towards solution of solid

dosage forms. Hardness can affect disintegration, so if the tablet is too hard it may not disintegrate within the required period of time. In this study, the tablets hardness was high, hence the need for disintegration test. All the tablets of the different brands of levofloxacin passed disintegration test (table 4). The various brands likely have disintegrants in the right proportion. The addition of disintegrants (e.g. starch, methyl cellulose) in the right proportion yields tablet products free of disintegration problems (Jantratid *et al*, 2008). Thus, the hardness of the tablets did not negatively affect the disintegration time of the tablets. Dissolution of drugs from oral solid dosage forms is a necessary criterion for drug bioavailability (i.e, the drug must be solubilized in the aqueous environment of the gastrointestinal tract to be absorbed). This is why dissolution test has emerged as one of the most important control tests for assuring product uniformity and batch-to-batch equivalence (Voegele, 1992). It is a useful index in predicting the probable *in vivo* performance of a drug as well as identifying unacceptable and poor-quality drug products (Shah, 2001; Jaman *et al*., 2015). The USP specifies that up to 80% of an immediate release dosage form is expected to dissolve in 30 minutes. All the brands, with the exception of C and E brands passed the test. The two brands had percentage drug releases of 75.96% and 74.34% respectively at 30 minutes (Figure 2). Two dissolution profiless are considered similar and bioequivalent if f1 is between 0 and 15 and f2 is between 50 and 100 (USFDA, 1997). The f1 and f2 values with respect to brand I, the innovator brand, is shown in table 5. Six brands, A, B, D, F, G and H gave f1 values between 0 and 15. Five brands, A, D, E, G and H gave f2 values between 50 and 100. Therefore, brands A, D, G and H can be used interchangeably with brand I, the innovator product. The spectrophotometric assay results showed that the various brands of levofloxacin are chemically equivalent because they had chemical content within the USP range of 90.0 %-110.0% (Table 6). The analytical method used for quantitative assay was very simple, rapid and cost-effective compared to most of the existing methods for the assay of levofloxacin. The method based on atomic absorption spectrophotometry (Salem *et al*, 2005) has a limitation of requiring expensive experimental set up. The chromatographic methods, though sensitive, are selective, and require complex and expensive instruments as well as demand for the provision and disposal of large quantities of solvents (Tuncel *et al*, 1992). The method also requires labour-intensive sample preparation procedure and personnel who are skilled in chromatography. The method based on condensation reaction (Sastry *et al*, 1995) involves boiling step for 20 minutes. Complexation reaction procedures require strict pH control (Chukwuenweniwe *et al*,1997) and is less sensitive. In contrast to the above published visible spectrophotometric methods, the extraction-free spectrophotometric method based on ion-pair complexation reaction requires only dyes and reagents which are cheap and readily available, and can be applied at ambient temperature, Colour development is instantaneous and neither involves strict pH adjustment nor complicated extraction steps. The method is sensitive and can be useful in quality control and routine analysis of levofloxacin in pharmaceutical dosage forms.

REFERENCES

Altiokka G, Atkosar Z, Can NO (2002). The determination of levofloxacin by flow injection analysis using UV detection, potentiometry, and conductometry in pharmaceutical preparations. *J Pharm Biomed Anal*., 30(3):881-5.

Chukwuenweniwe JE, Solomon OA, John OA (1997) *J. Antimicrobial Chemotherapy* 39:639-641

Cockburn R, Newton PN, Agyarko EK, Akunyili D, White NJ (2005) The Global Threat of Counterfeit Drugs: Why Industry and Governments Must Communicate the Dangers. PLoS Med 2(4): e100. https://doi.org/10.1371/journal.pmed.0020100 Retrieved 30/03/2020

Drlica K and Khao X. (1997) DNA gyrase, Topoisomerase IV and the 4-quinolones. *Microbial Molecular Biology.* 61:377-392.

Elbashir AA, Saad B, Ali AS, Saleh MI (2008). Development of a capillary electrophoresis method for the enantioselective estimation of primaquine in pharmaceutical formulations. *J AOAC Int*.; 91(3):536-41.

El-Brashy A.M., Metwally M.E, El-Sepai FA. (2004). Spectrophotometric determination of some fluoroquinolone antibacterials through charge-transfer and ion-pair complexation reactions. *Bulletin of the Korean Chemical Society* 25 (3): 365–372.

El-Brashy, A.M., Metwally, M.E, El-Sepai, F.A. (2005). Spectrophotometric determination of some fluoroquinolone antibacterial by ion-pair complex formation with cobalt (II) tetrathiocyanate. *Journal of the Chinese Chemical Society*, 52: 77–84.

Genazzani A, Pattarino F. (2008). Difficulties in production of identical products from a pharmaceutical technology viewpoint. *Drugs R D*, 9(2):65–72; doi:10.2165/00126839-200809020-00001

Igboasoiyi A.C., Eseyin O.A., Oladimeji H.O., Akpan RJ (2012). Quality Assessment of Some Brands of Piroxicam Capsules. *Nigerian Journal of Pharmaceutical and Applied Science Research*, 1(2): 91-97

Issa YM, Abdel-Gawad, FM, Table MA, Hussein H. (1997). Spectrophotometric determination of ofloxacin and levofloxacin hydrochloride with some sulphonphthalein dyes. *Analytical Letters* 30:2071–2084.

Jaman M, Chowdhury AA, Rana AA, Masum SM, Ferdous T, Rashid MA, Karim. MM (2015). *In vitro* evaluation of Ciprofloxacin Hydrochloride. *Bangladesh J. Sci. Ind. Res*. 50(4), 251-256.

Jantratid E., Janssen N, Reppas C, Dressman JB (2008) Dissolution media simulatingconditions in the proximal human gastrointestinal tract: an update. *Pharmaceutical Resources.* 25:1663-1676.

Joshi S (2002). HPLC separation of antibiotics present in formulated and unformulated samples. *Journal of Pharmaceutical and Biomedical Analysis*. 28(5):795-809

Meyyanathan SN, Ramasarma GV, Suresh B (2003). Analysis of levofloxacin in pharmaceutical preparations by high performance thin layer chromatography. *J. Sep. Sci.*, 26 (18) 1698–1700.

Moustafa ME, Amin SA, El-Dosoky RMS (2008). Spectrophotometric determination of some quinolone derivatives in dosage forms and biological fluids using ion-pair complex formation. *Analytical Letters.* 41: 837-852.

Nagaralli BS, Seetharamappa J, Melwanki MB (2002). Sensitive spectrophotometric methods for the determination of amoxycillin, ciprofloxacin and piroxicam in pure and pharmaceutical formulations. *J Pharm Biomed Anal*., 29(5):859-64.

Ofoefule, S. (2002). *A Textbook of Pharmaceutical Technology and Industrial Pharmacy, First* edition. Samikin Nigeria Ent. Lagos Nigeria. pp 58-65.

Ragab GH, Amin AS (2004). Atomic absorption spectroscopic, conductometric and colorimetric methods for determination of fluoroquinolone antibiotics using ammonium reineckate ion-pair complex formation. *Spectrochim Acta A Mol Biomol Spectrosc*., 60(4):973-8.

Salem HA (2005) Spectrophotometric, atomic absorption and spectrometric
determination of some fluoroquinolones". *American Journal of Applied Science*. 2:719-729.

Salem H., Fada L, Khater, W. (2007). Spectrofluorimetric determination of certain fluoroquinolones through charge transfer complex formation. *American Journal of Pharmacology and Toxicology*, 2:18–25.

Sastry CPS, Rao KR, and Prasad DS (1995). Spectrophotometric determination of enrofloxacin and ofloxacin in pharmaceutical formulations, *Indian Drugs*, 32(4):172–175.

Shah VP (2001). Dissolution: a quality control test vs a bioequivalent test. *Dissolution Technologies* 8(4): 1–2.

Tuncel M and Atkosar Z (1992) The determination of ofloxacin in tablets by potentiometry and conductometry, *Pharmazie*, 47(8):642–643.

United States Food and Drug Administration, Center for Drug Evaluation and Research (1997). Guidance for industry: Dissolution testing of immediate release solid oral dosage forms, available at: http://www.fda.gov/cder/Guidance/1713bp1.pdf retrieved 30/03/2020.

Voegele, D. (1992) "Drug release *in vitro*: An aid in clinical trials?" *Met. Find. Exp. Clinical Pharmacology*. 21:29-39.

World Health Organisation 19th WHO Model List of Essential Medicines, 2015; p.13.