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ORIGINAL ARTICLE

# Ion-pairing and reversed phase liquid chromatography for the determination of three different quinolones: Enrofloxacin, lomefloxacin and ofloxacin

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**KEYWORDS**

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Dosage forms

**Abstract** Two simple and sensitive high performance liquid chromatographic (HPLC) methods have been developed for the simultaneous determination of three different quinolones: enrofloxacin, lomefloxacin and ofloxacin in their pure and dosage forms, one with reversed phase HPLC and the other with ion-pair HPLC. In reversed phase HPLC, method (A), the mobile phase consists of 2.18% aqueous solution of $KH_2PO_4$ with pH adjusted to 2.4 ± 0.2 with acetonitrile (80:20; v/v), the mobile phase pumped at flow rate of 1.2 ml $min^{-1}$. A Neucleosil $C_{18}$ column (10 μm, 100 Å), 250 mm length × 4.6 mm diameter was utilized as stationary phase. Detection was affected spectrophotometrically at 294 nm. While in ion-pair HPLC, method (B), the mobile phase was aqueous solution of 0.65% sodium perchlorate and 0.31% ammonium acetate adjusted to pH 2.2 ± 0.2 with orthophosphoric acid: acetonitrile (81:19; v/v), the mobile phase pumped at flow rate of 1.5 ml $min^{-1}$. A $\mu$ bondapack $C_{18}$ column (10 μm, 100 Å), 250 mm length × 4.6 mm diameter was utilized as stationary phase. Detection was affected spectrophotometrically at 294 nm. Linearity ranges for enrofloxacin, lomefloxacin and ofloxacin were 4.0–108, 7.0–112 and 8.0–113 μg $ml^{-1}$, respectively using method A and 8.0–112, 7.0–112 and 5.0–105 μg $ml^{-1}$, respectively applying method B. Minimum detection limits obtained were 0.013, 0.023 and 0.035 μg $ml^{-1}$ for enrofloxacin, lomefloxacin and ofloxacin, respectively using method A, and 0.028, 0.023 and 0.011 μg $ml^{-1}$ using method B.

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The proposed methods were further applied to the analysis of enrofloxacin in injection and tablets containing the ofloxacin and lomefloxacin drugs, and the results were satisfied.



## 1. Introduction

Enrofloxacin (Enro), lomefloxacin (Lome) and ofloxacin (Oflo) are fluorinated 4-quinolone (Fig. 1) and have a wide spectrum of antibacterial activity (Monk and Campoli-Richards, 1987). Ofloxacin is among the fluoroquinolones considered promising for the treatment of ocular infections (Borrmann et al., 1988). An ophthalmic solution of ofloxacin was introduced for the topical treatment of ocular infections caused by susceptible gram-negative and gram-positive bacteria (Gwon, 1992). Analysis of fluoroquinolones in pharmacokinetic studies has rlied mainly on a variety of microbiological method (Wise et al., 1986) which are non-selective and imprecise compared with more recent approaches using high performance liquid chromatography (HPLC) (Basci et al., 1996; Nemutlu et al., 2007; Suna et al., 2007). Selective determination of flouroquinolone derivatives from tablets by reverse-phase was investigated (Shinde et al., 1998; Marilyn et al., 2007; Espinosa-Mansilla et al., 2005). Quantitative determination of Enro, Lome and Oflo in pharmaceutical dosage, bulk drugs and process monitoring of enrofloxacin by RP-HPLC was studied (Argekar et al., 1996). A simple, rapid and sensitive HPLC method was developed for the assay of Enro in raw material and injection (Souza et al., 2002; Salehzadeh et al., 2007). The fate of Enro present in raw sewage at a swine-breeding facility was investigated by liquid–liquid extraction and reversed phase liquid chromatography with photodiode array detection (Pierini et al., 2004). Determination of a series of quinolone antibiotics using liquid chromatography–mass spectrometry was studied (Ballesteros et al., 2004; Santoro et al., 2006). A rapid and simple procedure for determination of enrofloxacin and ciprofloxacin in bovine milk and plasma is described (Idowu and Peggins, 2004). The aim of the present work is to develop a simple, rapid, sensitive and reliable HPLC assay procedures to quantify ofloxacin, lomefloxacin and enrofloxacin in their pharmaceutical dosage forms.

## 2. Experimental

### 2.1. Apparatus

Chromatographic separation and detection was performed on high performance liquid chromatography (HPLC) system which consisted of pump (WATERS Model 515), an autosampler (WATERS Model 717) and a Dual $\lambda$ absorbance detector (WATERS model 2489) with 10 mm path length cell. The data were recorded on a personal computer, using the manufacturer software package (Millennium 32, Version 3.02, WATERS). A Jenway Instruments (Germany) pH meter was used for pH control; the instrument has previously been calibrated against standard buffer solutions of pH 2.0, 4.0 and 7.0.

### 2.2. Drugs

Enrofloxacin (99.92%), lomefloxacin hydrochloride (99.95%) and ofloxacin (99.52%) were kindly supplied by Egyptian International Pharmaceutical Industries Company (EIPICO), Egypt, Pharmaceutical dosage forms were bought from local market.

### 2.3. Reagents

- All reagents were obtained from VWR Chemicals (Pool, England).
- Methanol (HiPerSolv for HPLC).
- Acetonitrile (HiPerSolv).
- Potassium dihydrogen phosphate (AnalaR).
- Orthophosphoric acid (about 85%, AnalaR), were obtained from VWR Chemicals (Pool, England).
- Millipore 0.45 μm nylon membrane filter (USA).
- Sodium perchlorate (GPR) and ammonium acetate crystals (AnalaR).
- The high purity water was prepared using WATERS Ultra pure water system (WATERS, USA).

### 2.4. Solution preparations

#### 2.4.1. Stock and working standards solutions

– Enro, Lome and Oflo stock solutions containing 1.0 mg $ml^{-1}$ of each in methanol were prepared separately by weighing 100 mg each of Enro, Lome and Oflo in 100 ml volumetric flask and diluted to the mark with the same solvent (standards stock solutions).
– Working standards solutions of Enro, Lome and Oflo were prepared separately by diluting 5.0 ml from each standard stock solution, to 100 ml with mobile phase A or B for

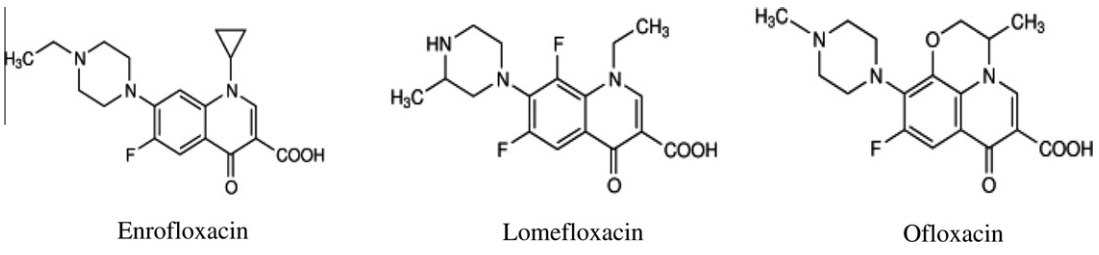


**Figure 1** The chemical structure of three pure drug materials.

methods A or B, respectively. A portion of resulted solution was filtered through 0.45 μm membrane filter.

*2.4.2. Preparation of buffer solutions*

A. *Phosphate solution:* A 21.8 g of potassium dihydrogen phosphate was dissolved in 1000 ml of purified water and the pH was adjusted to 2.4 with orthophosphoric acid (~85%).
B. *Perchlorate solution:* A 3.1 g of ammonium acetate and 6.5 g of sodium perchlorate were dissolved in 1000 ml of purified water. The pH of resulted solution was adjusted to 2.2 with orthophosphoric acid (~85%).

*2.5. Application of pharmaceutical dosage forms*

*2.5.1. For method (A) reversed phase HPLC*

*Tablets:* 20 tablets were weighed and finely powdered. To a quantity of powdered tablets equivalent 200 mg of Lome or 200 mg Oflo, 150 ml of methanol were added and dispersed with aid of sonication for 5 min, shacked for 15 min. A sufficient of methanol was added to produce 200 ml, mixed. The mixture was centrifuged and 5.0 ml of supernatant liquid were diluted to 100 ml with mobile phase A. A portion or resulted solution was filtered through 0.45 μm membrane filter.

*Injection:* Accurately, 2.0 ml of injection were transferred to 200 ml volumetric flask, mixed with 150 ml of methanol and sonicated for 5.0 min. A sufficient of methanol was added to produce 200 ml, mixed. Resulted solution (5.0 ml) were diluted to 100 ml with mobile A. A portion of resulted solution was filtered through 0.45 μm membrane filter.

*2.5.2. For method (B) ion-paring mobile phase*

As preparation with method (A) but the second dilution was performed with mobile phase (B). The methods were applied for analysis of more than one type of tablets and injections purchased from local marketing and the results were listed in Table 6.

*2.6. Chromatographic conditions*

*2.6.1. Method (A) reversed phase HPLC*

The chromatographic separation was performed on a $C_{18}$ column (10 μm, 100 Å), 250 mm length × 4.6 mm diameter particle size, Neucleosil $C_{18}$ reversed-phase column packed with

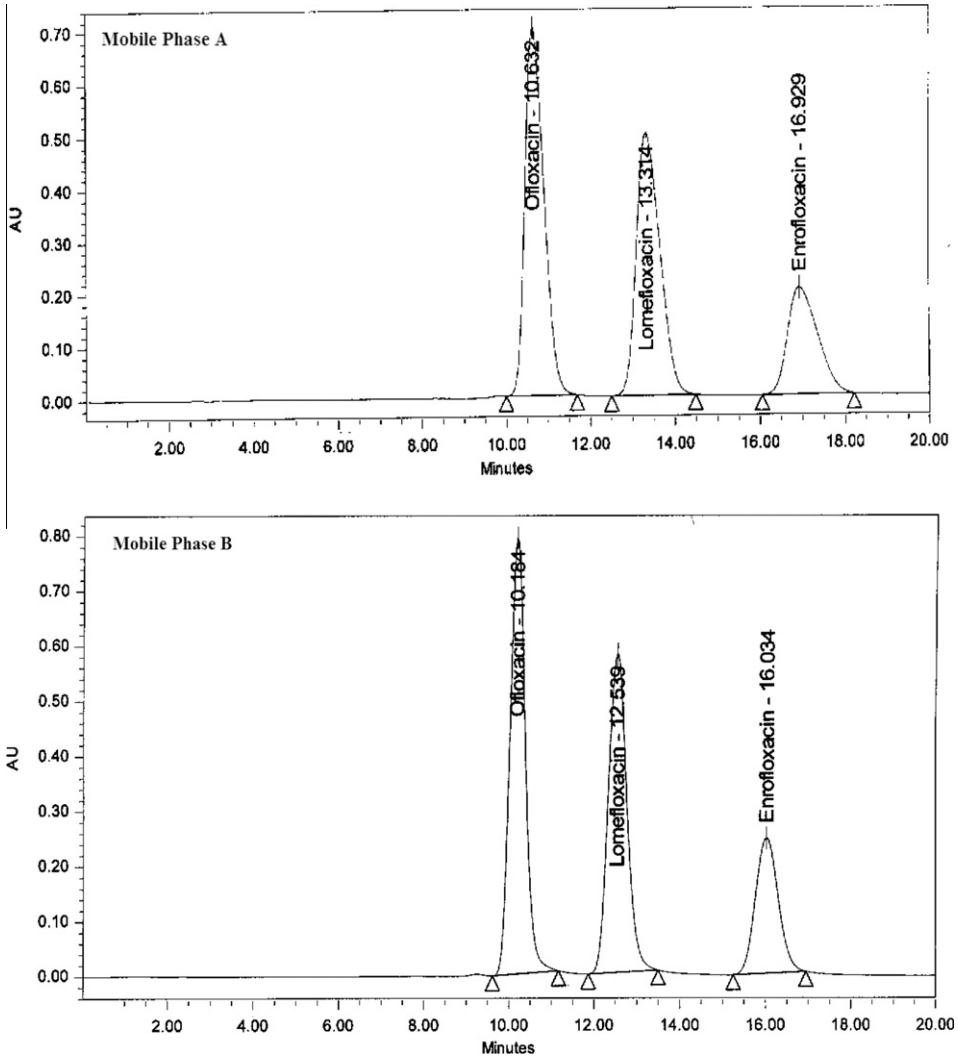


**Figure 2** Separation of Enro, Lome and Oflo – A – mobile method (A) and mobile method (B).

dimethyloctadecyl-silyl bonded amorphous silica. The mobile phase was consists of 2.18% aqueous solution of $KH_2PO_4$ with pH adjusted to 2.4 ± 0.2 with acetonitrile (80:20; v/v), the mobile phase pumped at flow rate of 1.2 ml $min^{-1}$ the mobile phase was filtered through 0.45 μm nylon filter, degassed for 15 min then pumped at flow rate 1.2 ml $min^{-1}$. The column was kept at 20.0 ± 2.0 °C during the analysis; the detection wavelength was 294 nm and the injection volume was 20 μl.

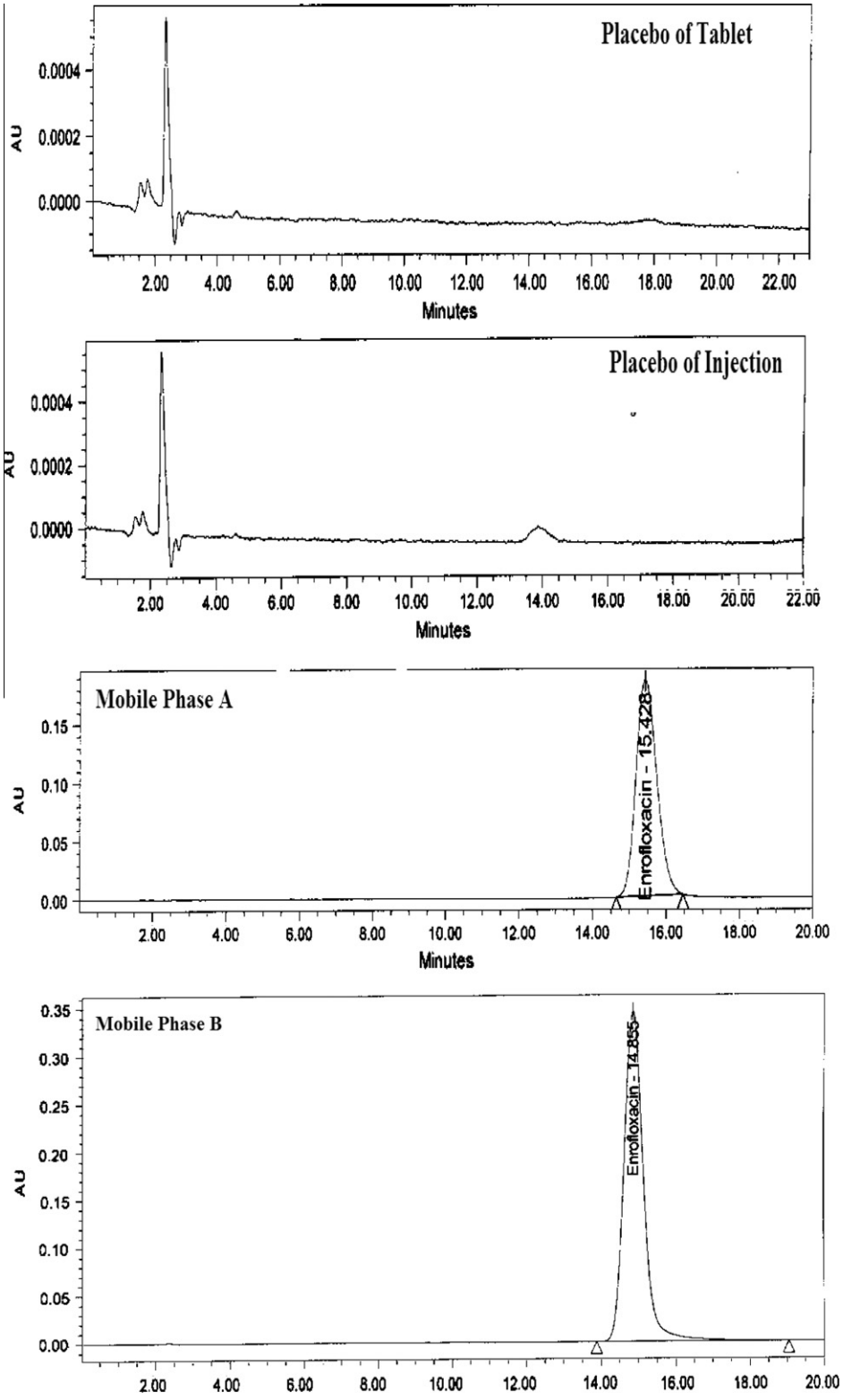


**Figure 3** Chromatograms obtained during selectivity (cont.).

*2.6.2. Method (B) Ion-pairing HPLC*

The mobile phase was aqueous solution of 0.65% sodium perchlorate and 0.31% of ammonium acetate adjusted to pH 2.2 ± 0.2 with orthophosphoric acid:acetonitrile (81:19; v/v). The mobile phase was filtered through 0.45 μm nylon membrane filter, degassed for 15 min then pumped at flow rate was 1.5 ml $min^{-1}$. The column was kept at 20.0 ± 2.0 °C during the analysis; the detection wavelength was 294 nm and the injection volume was 20 μl. A $\mu$ bondapack $C_{18}$ column (10 μm, 100 Å), 250 mm length × 4.6 mm diameter was utilized as stationary phase. Detection was affected spectrophotometrically at 294 nm.

## 3. Results and discussions

The cationic nature of Enro, Lome and Oflo leads to broad asymmetric peaks in RP-HPLC with aqueous-organic mobile phases and conventional $C_{18}$ columns, because of the ionic interaction with the alkyl-bonded reversed-phase packing. Also using of methanol or acetonitrile lead to unresolved and high tailing peaks.

### *3.1. Method (A) reversed phase HPLC*

In order to affect the simultaneous elution of three peaks under isocratic conditions the mobile phase composition (organic modifier, flow rate, ionic strength and pH) has been investigated. The reversed phase HPLC method (A) proposed using of low pH solution with acetonitrile to block the residual silanol interaction and reduce tailing, orthophosphoric acid is used here in pH adjustment. The mobile phase (A) was consists of 2.18% aqueous solution of $KH_2PO_4$ with pH adjusted to 2.4 ± 0.2 with acetonitrile (80:20; v/v). The mobile phase compositions were optimized by changing the pH range (2.0–3.0) in addition to changing temperature (20–30 °C). Under the described conditions, the three components were defined, resolved and free from tailing, the tailing factors were $<1.3$ for all peaks. The elution order were Oflo ($t_R$ = 10.6), Lome ($t_R$ = 13.3) and Enro ($t_R$ = 16.9) (Fig. 2).

### *3.2. Method (B) Ion-paring mobile phase*

An ion-pairing substance as sodium perchlorate solution with acetonitrile was used to decrease the residual silanol interaction and decrease tailing, the mobile phase was an aqueous solution of 0.65% sodium perchlorate and 0.31% of ammonium acetate adjusted to pH 2.2 ± 0.2 with orthophosphoric acid: acetonitrile (81:19; v/v), the mobile phase composition were optimized by changing the pH range (2.0–3.0) in addition to temperature changed from 20–30 °C. Under the described conditions the three components were well defined, resolved and free from tailing, the tailing factors were $<1.2$ for all peaks. The elution order were Oflo ($t_R$ = 10.2), Lome ($t_R$ = 12.5) and Enro ($t_R$ = 16.0), (Fig. 2).

### *3.3. Validity of the methods*

*3.3.1. Selectivity*

For chromatographic methods, selectivity is the ability of the method to accurately measure the analyte in presence of all potential sample components. The selectivity of both methods was checked by two ways, the first way by comparison the chromatograms obtained from dosage forms samples and the corresponding placebo (Fig. 3). It is clear that there is no significant peak in the placebo chromatogram at retention time of three separated peaks is appeared in the test chromatogram. The second way to check the selectivity of both methods was standard addition method in which a known concentration of the analyte were added to previously analyzed pharmaceutical preparation (Table 7).

*3.3.2. Linearity*

The linear correlation between area under peaks and compound concentrations was checked for each component using both methods. Data for six different concentrations was ranged in (4.0–108 μg $ml^{-1}$) for Enro, (7.0–112 μg $ml^{-1}$) for Lome and (8.0–113 μg $ml^{-1}$) for Oflo with method (A), and (8.0–112 μg $ml^{-1}$) for Enro, (7.0–112 μg $ml^{-1}$) for Lome, and (5.0–105 μg $ml^{-1}$) for Oflo with method (B) were collected and analyzed. Each solution was injected for five times then the least square method was used for calculation of the slope, intercept and correlation coefficient ($r$) for compounds with both mobile phases. The correlation between the analyte concentration and peak area is described by linear regression equations with high value of correlation coefficient ($r$) all results were listed in Table 1.

*3.3.3. Limit of detection and limit of quantification*

The limits of detection and of quantification were calculated in accordance with 3.3 s/m and 10 s/m criteria (ICH, 1995), respectively. Where s is standard deviation of peak area (for five replicates) for analyte and (m) is the slope of calibration

**Table 1** Characteristics of the proposed methods used in assay of Enro, Lome and Oflo.

| Parameter | RP-HPLC (method A) | | | Ion-pairing (method B) | | |
|---|---|---|---|---|---|---|
| | Enro | Lome | Oflo | Enro | Lome | Oflo |
| Linearity range/μg $ml^{-1}$ | 4.0–108 | 7.0–112 | 8.0–113 | 8.0–112 | 7.0–112 | 5.0–105 |
| Slope | $2.16 \times 10^3$ | $7.5 \times 10^3$ | $16.5 \times 10^3$ | $5.4 \times 10^3$ | $0.5 \times 10^3$ | $1.2 \times 10^3$ |
| Intercept (a) | $11.5 \times 10^3$ | $3.9 \times 10^3$ | $4.7 \times 10^3$ | $1.7 \times 10^3$ | $3.1 \times 10^3$ | $3.8 \times 10^3$ |
| Correlation coefficient | 0.99994 | 0.99960 | 0.99990 | 0.99997 | 0.99942 | 0.99988 |
| Detection limit/μg $ml^{-1}$ | 0.013 | 0.023 | 0.035 | 0.028 | 0.023 | 0.011 |
| Quantification limit/μg $ml^{-1}$ | 0.044 | 0.077 | 0.117 | 0.094 | 0.077 | 0.037 |
| Capacity factor | 15.92 | 12.31 | 9.63 | 15.30 | 11.53 | 9.18 |
| Tailing factor | 1.25 | 1.25 | 1.32 | 1.18 | 1.19 | 1.17 |
| Theoretical plate no. | 3272 | 2804 | 2649 | 4232 | 4001 | 3889 |

Regression equation = $A = a + bC$, where $A$ is the area under peak and $a$, is the intercept.

**Table 2** Evaluation of accuracy of the proposed methods.

| Drugs | Theoretical conc. | RP-HPLC method A | | | Ion-pairing method B | | |
|---|---|---|---|---|---|---|---|
| | | Found[a] | Recovery | RSD% | Found[a] | Recovery | RSD% |
| Enro | 15.03 | 14.97 | 99.6 | 0.25 | 15.21 | 101.2 | 0.21 |
| | 40.08 | 40.27 | 100.5 | 0.25 | 39.93 | 99.6 | 0.50 |
| | 45.09 | 45.38 | 100.7 | 0.68 | 45.55 | 101.0 | 0.48 |
| | 50.10 | 50.49 | 100.8 | 0.10 | 50.99 | 101.8 | 0.36 |
| | 55.11 | 54.59 | 99.1 | 0.04 | 55.15 | 100.1 | 0.25 |
| | 87.68 | 88.65 | 101.1 | 0.23 | 88.36 | 100.8 | 0.27 |
| Lome | 14.94 | 14.89 | 99.7 | 0.47 | 15.01 | 100.5 | 0.11 |
| | 39.84 | 40.18 | 100.8 | 0.40 | 39.66 | 99.6 | 0.75 |
| | 44.82 | 45.45 | 101.4 | 0.10 | 45.05 | 100.5 | 0.33 |
| | 49.80 | 49.57 | 99.5 | 0.70 | 50.05 | 100.5 | 0.40 |
| | 54.78 | 54.81 | 100.1 | 0.36 | 54.81 | 100.1 | 0.67 |
| | 87.15 | 88.68 | 101.8 | 0.81 | 87.93 | 100.9 | 0.67 |
| Oflo | 15.18 | 15.23 | 100.3 | 0.63 | 15.07 | 99.3 | 0.10 |
| | 40.48 | 40.76 | 100.7 | 0.60 | 40.62 | 100.3 | 0.63 |
| | 45.54 | 45.62 | 100.2 | 0.58 | 45.49 | 99.9 | 0.55 |
| | 50.60 | 50.19 | 99.2 | 0.43 | 50.84 | 100.5 | 0.33 |
| | 55.66 | 56.19 | 101.0 | 0.36 | 55.44 | 99.6 | 0.57 |
| | 88.55 | 88.24 | 99.7 | 0.10 | 88.96 | 100.5 | 0.40 |

[a] Average of five determinations.

plot, determination from the linearity investigation. The LOD and LOQ obtained were listed in Table 1.

### 3.4. Accuracy

In order to determine the accuracy of the proposed methods solution contains six different concentration of Enro, Lome and Oflo were prepared and analyzed. The recoveries obtained among studied concentrations range of each Enro, Lome and Oflo were 100.28 ± 0.78%, 100.54 ± 0.93% and 100.17 ± 0.66% with mobile phase A and 100.75 ± 0.78%, 100.33 ± 0.46% and 100.01 ± 0.49% with mobile phase B, respectively. The results obtained from these investigations were summarized in Table 2.

### 3.5. Precision

The precision of the methods expressed as the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Repeatability and reproducibility expressed as RSD% were characterized by spread data from replicate determination. The intra-day precision (repeatability) of both methods were evaluated by analysis by the mean of five replicates of three different reference standard solution containing 80%, 100% and 110% of the labeled amount of Enro (100 mg/ml) in Avitryl injection, Lome (400 mg/tablet) in Lomax tabs and Oflo (200 mg/tablet) in Oflicin tabs. The inter-day precision (reproducibility) of both

**Table 3** Summary of repeatability (intra-day) and reproducibility (inter-day) precision data for Enro, Lome and Oflo with mobile phase (A) and mobile phase (B).

| Theoretical conc. ($\mu g\ ml^{-1}$) | Intra-day | | Inter-day amount recovered ± RSD | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Recovery ± RSD | | After 2 days | | After 4 days | | After 8 days | |
| | A | B | A | B | A | B | A | B |
| *Enrofloxacin* | | | | | | | | |
| 40.16 | 100.98 ± 0.42 | 100.43 ± 0.41 | 101.00 ± 0.81 | 100.20 ± 0.20 | 100.78 ± 0.52 | 99.46 ± 0.58 | 99.80 ± 0.23 | 98.96 ± 0.23 |
| 50.20 | 100.97 ± 0.13 | 101.27 ± 0.13 | 101.45 ± 0.24 | 100.78 ± 0.79 | 101.15 ± 0.17 | 100.78 ± 0.21 | 99.13 ± 0.89 | 99.65 ± 0.64 |
| 55.22 | 99.84 ± 0.87 | 100.24 ± 0.74 | 99.47 ± 0.77 | 100.34 ± 0.14 | 99.21 ± 0.19 | 100.45 ± 0.22 | 98.47 ± 0.66 | 98.32 ± 0.68 |
| *Lomefloxacin* | | | | | | | | |
| 39.92 | 100.64 ± 0.91 | 100.31 ± 0.10 | 99.91 ± 0.43 | 100.24 ± 0.14 | 100.14 ± 0.31 | 99.75 ± 0.26 | 99.41 ± 0.48 | 98.87 ± 0.65 |
| 49.90 | 99.49 ± 0.98 | 100.01 ± 0.28 | 99.61 ± 0.60 | 100.09 ± 0.23 | 99.14 ± 0.30 | 99.93 ± 0.14 | 98.82 ± 0.04 | 99.02 ± 0.45 |
| 54.89 | 100.54 ± 0.76 | 100.26 ± 0.45 | 99.94 ± 0.16 | 99.68 ± 0.23 | 100.04 ± 0.47 | 99.83 ± 0.18 | 98.45 ± 0.24 | 98.94 ± 0.24 |
| *Ofloxacin* | | | | | | | | |
| 40.24 | 100.45 ± 0.31 | 100.04 ± 0.13 | 100.32 ± 0.22 | 100.73 ± 0.16 | 100.14 ± 0.12 | 100.49 ± 0.53 | 99.45 ± 0.33 | 99.19 ± 0.61 |
| 50.30 | 99.34 ± 0.55 | 99.89 ± 0.26 | 100.39 ± 0.82 | 100.67 ± 0.37 | 99.83 ± 0.25 | 99.56 ± 0.50 | 98.21 ± 0.53 | 98.33 ± 0.55 |
| 55.33 | 100.69 ± 0.17 | 100.73 ± 0.39 | 100.98 ± 0.25 | 100.52 ± 0.13 | 100.16 ± 0.33 | 100.12 ± 0.27 | 99.08 ± 0.19 | 98.59 ± 0.57 |

methods were evaluated by analysis of freshly prepared reference solution (second dilution; i.e. 100%) of the labeled amount on four different days and results obtained from this analysis were listed in Table 3 as mean recovery (%). The results showed that there is no difference either within day or between days implying that the repeatability and reproducibility of both methods was good. The results obtained were compared statistically by Student's *t*-test (for accuracy), and variance ratio *F*-test (for precision) (Miller and Miller, 2000), at 95% confidence level. The results showed that the *t*- and *F*-values were lower than the critical values indicating that there was no significant difference between the proposed methods.

### 3.6. Robustness

The methods were found to be robust although small deliberate changes in method conditions did have some effect on the chromatographic behavior of the solutes. The chromatographic condition investigated was mobile phase composition, pH, flow rate, column temperature and detected wavelength. The results of robustness analysis showed that although small changes in the mobile phase pH has no significant effect on the retention time for Enro, Lome and Oflo with both methods. The changes in the flow rate, leading to increase in the peak tailing and increase the broadening of analyte peaks (Table 4). Changing in the column temperature has larger effect on the chromatographic behavior of the three peaks with both methods than mobile phase, pH and flow rate. While the reduction in the concentration of sodium perchlorate in method (B) leads to partial deterioration in the behavior of three solutes. Also the decrease in the concentration of buffer solution in mobile phase (A) lead to deterioration of the chromatographic behavior of both solutes. Finally altering of the wavelength detection has no effect on the chromatographic behavior of both solutes with the two methods.

### 3.7. System suitability

According to USP (The United State Pharmacopoeia, 2007), system suitability tests are an integral part of liquid chromatographic method. System suitability tests are used to verify that resolution and reproducibility were adequate for analysis performed. The parameters of this test are column efficiency (number of theoretical plates), asymmetry of chromatographic peak, peak resolution factor, and repeatability as RSD of peak area for five injections and reproducibility of retention as RSD of retention time. The results of these tests and their acceptance criteria according to USP regulation were listed in Table 5. From the obtained results on comparison with the specification set for the methods can be used to draw conclusion about the suitability of system for analysis.

### 3.8. Analytical applications

Pharmaceutical formulations containing Enro, Lome and Oflo were analyzed successfully by the proposed method with a good recovery. Results are recorded in Table 6 confirming that the proposed method is not liable to interference by injection

**Table 4** Effect of column temperature and mobile phase pH on the chromatographic behavior of Enro, Lome and Oflo with method A and B.

| Parameters | Mobile phase | | | | | Column temperature | | |
|---|---|---|---|---|---|---|---|---|
| | 2.0 | 2.2 | 2.4 | 2.6 | 3.0 | 20 °C | 25 °C | 30 °C |
| Method A: RP-HPLC | | | | | | | | |
| *Enrofloxacin* | | | | | | | | |
| *K* | 15.50 | 15.87 | 15.92 | 15.94 | 16.31 | 15.92 | 15.96 | 15.43 |
| As | 1.27 | 1.25 | 1.25 | 1.31 | 1.35 | 1.25 | 1.20 | 1.20 |
| *N* | 3232 | 3282 | 3272 | 3255 | 3200 | 3272 | 3266 | 3221 |
| *Lomefloxacin* | | | | | | | | |
| *K* | 12.00 | 12.29 | 12.31 | 12.34 | 12.88 | 12.31 | 12.23 | 11.88 |
| As | 1.32 | 1.15 | 1.25 | 1.26 | 1.35 | 1.25 | 1.23 | 1.27 |
| *N* | 2700 | 2844 | 2804 | 2837 | 2577 | 2804 | 2824 | 2800 |
| *Ofloxacin* | | | | | | | | |
| *K* | 9.57 | 9.60 | 9.63 | 9.65 | 9.78 | 9.63 | 9.58 | 9.44 |
| As | 1.29 | 1.33 | 1.32 | 1.34 | 1.35 | 1.32 | 1.33 | 1.40 |
| *N* | 2600 | 2635 | 2649 | 2637 | 2599 | 2649 | 2630 | 2588 |
| Method B: Ion-pair-HPLC | | | | | | | | |
| *Enrofloxacin* | | | | | | | | |
| *K* | 15.27 | 15.30 | 15.22 | 15.66 | 15.78 | 15.30 | 15.49 | 15.86 |
| As | 1.20 | 1.18 | 1.20 | 1.23 | 1.29 | 1.18 | 1.20 | 1.29 |
| *N* | 4230 | 4232 | 4221 | 4189 | 4100 | 4232 | 4225 | 4155 |
| *Lomefloxacin* | | | | | | | | |
| *K* | 11.50 | 11.53 | 11.47 | 11.66 | 11.79 | 11.53 | 11.55 | 12.55 |
| As | 1.18 | 1.19 | 1.20 | 1.25 | 1.21 | 1.19 | 1.22 | 1.20 |
| *N* | 3885 | 4001 | 3985 | 3975 | 3844 | 4001 | 3900 | 3891 |
| *Ofloxacin* | | | | | | | | |
| *K* | 9.08 | 9.18 | 9.23 | 9.29 | 9.77 | 9.18 | 9.22 | 9.79 |
| As | 1.18 | 1.17 | 1.20 | 1.29 | 1.21 | 1.17 | 1.12 | 1.10 |
| *N* | 3885 | 3889 | 3875 | 3537 | 3493 | 3889 | 3879 | 3895 |

*K*, capacity factor; *N*, no. of theoretical plates; As, asymmetry factor.

**Table 5** Summary of system suitability tests.

| Parameter | Method A | | | Method B | | |
|---|---|---|---|---|---|---|
| | Enro | Lome | Oflo | Enro | Lome | Oflo |
| *K* | 15.92 | 12.31 | 9.63 | 15.30 | 11.53 | 9.18 |
| *R*[b] | 3.0 | 2.8 | 2.8 | 3.8 | 3.2 | 3.2 |
| *N* | 3272 | 2804 | 2649 | 4232 | 4001 | 3884 |
| As | 1.25 | 1.25 | 1.32 | 1.18 | 1.19 | 1.17 |
| RSD[a] (peak areas) | 0.79 | 0.68 | 0.65 | 0.82 | 0.76 | 0.51 |
| RSD[a] (retention time) | 0.29 | 0.42 | 0.42 | 0.24 | 0.17 | 0.19 |

*K*, capacity factor; *N*, no. of theoretical plates; *R*, resolution factor; As, asymmetry factor.
[a] RSD for five determinations.
[b] The resolution factor (*R*) calculated to the nearest peak in order.

**Table 6** Determination of Enro, Lome and Oflo in pharmaceutical dosage forms.

| Drugs | Company | Active | Labeled (mg) | Method A | | | Method B | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Found[a] (mg) | Recovery (%) | RSD (%) | Found[a] (mg) | Recovery (%) | RSD (%) |
| Enrocxin 10% Inj | Alexandria | Enro | 100 mg/ml | 101.80 | 101.80 | 0.56 | 101.46 | 101.46 | 0.63 |
| Avitryl 10% Inj | AVICO | Enro | 100 mg/ml | 102.47 | 102.57 | 0.24 | 102.15 | 102.15 | 0.47 |
| Lomex Tab | Sigma | Lome | 400 mg | 404.04 | 101.01 | 0.49 | 404.80 | 101.20 | 0.75 |
| Lomax Tab | Julphar | Lome | 400 mg | 401.48 | 100.37 | 0.2 | 399.96 | 99.99 | 0.34 |
| Tarivid Tab | Hoechst | Oflo | 200 mg | 200.32 | 100.16 | 0.84 | 200.80 | 100.40 | 0.66 |
| Oflicin | Memphis | Oflo | 200 mg | 203.22 | 101.61 | 0.65 | 201.88 | 100.94 | 0.46 |

[a] Average of five determinations.

**Table 7** Determination of Enro, Lome and Oflo in pharmaceutical dosage forms applying the standard addition technique.

| Dosage form | Taken (μg/ml) | Added (μg/ml) | Method A | | Method B | |
|---|---|---|---|---|---|---|
| | | | Found[a] (μg/ml) | Recovery (%) | Found[a] (μg/ml) | Recovery (%) |
| Enrocxin 10% Inj (Enro) | 20.36 | 20.06 | 40.18 | 99.4 | 40.10 | 99.2 |
| | | 30.09 | 50.83 | 100.8 | 51.00 | 101.1 |
| | | 40.12 | 60.60 | 100.2 | 60.99 | 100.9 |
| Lomax Tab (Lome) | 20.07 | 20.02 | 39.93 | 99.6 | 40.44 | 100.9 |
| | | 30.03 | 50.45 | 100.7 | 50.71 | 101.2 |
| | | 40.04 | 61.02 | 101.5 | 59.57 | 99.1 |
| Oflicin (Oflo) | 20.32 | 20.08 | 40.64 | 100.6 | 39.96 | 98.9 |
| | | 30.12 | 51.20 | 101.5 | 50.85 | 100.8 |
| | | 40.16 | 60.24 | 99.6 | 61.45 | 101.6 |

[a] Average of five determinations.

and tablet fillers, excipients and additives usually formulated with injection and tablets. The standard addition method in which a known concentration of the analyte were added to previously analyzed pharmaceutical preparation (Table 7). The proposed method is highly sensitive; therefore, it could be used easily for the routine analysis of pure form and in its pharmaceutical formulations.

## 4. Conclusion

Two simple, sensitive, accurate, reproducible and precise liquid chromatographic methods for assay of Enro, Lome and Oflo in pure and bulk forms have been developed and validated. The advantages of the proposed methods are lower detection limits and higher quantification limit this permit wide range of analysis. Furthermore, these methods are capable to determine one of the three components in the presence of other two.

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