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Hollow Fiber Nano Membrane as Liquid Phase Microextraction for Determination of Enrofloxacin in the Prsence of Florfenicol and Tylosin in Chicken Tissues

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Abstract

Veterinary medications are a diverse class of medicines with a wide range of chemical and therapeutic qualities. Food items obtained from treated animals may contain residues of the parent chemicals, their metabolites, and/or conjugates due to the usage of veterinary medical treatments in food-producing animals. Enrofloxacin is the most often used fluoroquinolone in poultry disease management and treatment. Because of the limited bioavailability of fluoroquinolones, they are mostly excreted as unaltered molecules in feces and urine, and so discharged into the environment, resulting in significant levels of enrofloxacin residues. For sample preconcentration, a polypropylene hollow fiber membrane with a 200 nm pore size, 600 µm internal diameter, and 200 µm wall thickness was utilized as liquid phase microextraction. Under the optimizations of two variables of divisor concentration and working wavelength Enrofloxacin was determined in concentration ranges of 0.1- 25 µg/mL at 270 and 272 nm using successive ratio- derivative technique with a limit of detections 0.024, 0.042 µg/mL, and r^2 equal to 0.9993, 0.9983.The present method was successfully used for the determination of enrofloxacin in the presence of florfenicol and tylosin and it was applied for quantification of enrofloxacin in chicken samples with recovery ranged from 95.33 - 99.66%.

Keyword Hollow fiber, Nano membrane, Microextraction, Enrofloxacin, Florfenicol and Tylosi, Successive Derivative Ratio, Chicken tissues

1. Introduction

Veterinary medications are a diverse class of medicines with a wide range of chemical and therapeutic qualities. Food items obtained from treated animals may contain residues of the parent chemicals, their metabolites, and/or conjugates due to the usage of veterinary medical treatments in food-producing animals. Excessive use of veterinary antibiotics, for example, has raised concerns about a rise in allergies and antimicrobial-resistant microbes. Other medications have been banned because of their proven carcinogenicity (e.g., diethylstilbestrol, nitrofurans, and chloramphenicol)(1). Earlier to the instrumental investigation, test arrangement is ordinarily required to disconnect and pre-concentrate the analytes. Fluid stage microextraction, also known as hollow fiber membrane microextraction, attempts to increase extraction selectivity while reducing the amount of organic solvents and glassware needed. Mmbbrane technology had grat success in most types which utilizes level sheet and empty fiber manufactured natural layers, is getting to be progressively utilized in medication, science, chemistry, biotechnology, water treatment, and the environment due to its multidisciplinary nature (2, 3). The segment coefficient of analyte (s) between the bulk test and the extricated stage decides the extraction abdicate in microextraction. Because partitioning is independent of analyte concentration, sample concentration can be determined using the absolute amount extracted. (4).

The analytes are extracted little volume of organic solvents (usually 1–100 µL) in Liquid-phase micro extraction methods.

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An idle permeable capillary developed utilized as a carrier for the extraction dissolved substance within the halo fibre Liquid-Phase Microextraction (HF-LPME). It has two modes of operation. Two-phase halo fibre Liquid-Phase Microextraction (HF-LPME).. In this strategy, an organic solvent fills the pores and lumen of a semipermeable layer, which may be a permeable empty fiber made e.g. of polypropylene. After inductingthe membrane in the sample lest he segment through the pores of the film into interior organic solvent inter. Three-phase halo fibre liquid-phase microextraction. In this procedure, an immiscibler solvent fills the pores of the hallow fiber, which isolated from tested sample another solvente interior the lumen, so that two equilibria for the analytes take put one between the watery test and the natural dissolvable within the capillary divider pores, and the other between the solvent within the pores and thesolvent within the lumen. In other words, the analyte cross the natural solvent implanted within the gaps of the semipermeable film and concentrated into in ther third stage the capillary's lumen in fish muscle, dregs, water (5). chicken meat(6). milk(7). chicken,pork, swine liver, swine kidney, pharmaceutical formulation by (8, 9). in dog urine, but no work utilized halo fibre liquid-pPhase microextraction for determination of enrofloxacin In the present work enrofloxacin was determined in by halo fibre liquid-pPhase microextraction using successive derivative ratio spectra. (10)The method is based on the successive derivatives of ratio spectra in two steps. The minimum or maximum of the first derivative third ratio spectra with respect to wavelength was chosed for determination of enrofloxacin. (11, 12)During the extraction, the desired analyte under proper con¬ditions is first transferred into the organic phase and then into the acceptor phase. The rate of conducting the extraction depends on the rate of mass transfer between the two interfaces of the donor/organic phase and the organic/acceptor phase. The main reported limitation of this method is that the method only applicable for species which are capable of ionization [4].

2. Experimental

2.1. Apparatus

The absorbance spectra were done by a double beam UV-Vis spectrophotometer (UV-1800, Shimadzu, Japan) – usinng quartz cell and connected to a computer loaded with the software UV Probe program.

Microsyringe: 50µL Hamiltone syringe (Bondaduz, Switzerland)

Hollow fiber membrane: Q3/2 Accrual polypropylene hollow fiber membrane from Membrana (Wuppertal, Germany) with a 0.2 µm pore size, 600 µm internal diameter and 200 µm wall thickness.

Scanning electron microscope (SEM): the measurements of scanning electron microscope were carried out at Selçuk Üniversitesi/ Konya/ Turkey)

2.2. Preparation of Hollow Fiber Membrane

The hollow fiber was cut into 10.5 cm long pieces, cleaned in acetone to eliminate any contamination, and dried at room temperature. A syringe was filled with a specified amount of acceptor phase fluid (about double the volumes of the hollow fiber piece's lumen), and the tip of the syringe was placed into one end of the hollow fiber piece. The organic solvent was dipped cutinto the hollow fiber to penetrate (impregnate) the organic solvent into the hollow fiber pores. (13, 14). After the organic solvent has been trapped in the HF pores by capillary forces and hydrophobic interactions, the plunger of the syringe is squeezed to force the acceptor phase (24 µL) out of the syringe and into the hollow fiber lumen. The excess acceptor phase solvent escapes via the fiber lumen, flushing any organic phase from the hollow fiber lumen. Finally, a soldering iron is used to close the opposite end of the HF (15).

2.3. Reagents

All reagents used throughout this study are of analytical grad. Enrofloxacin, Florfenicol, Tylosin (Sigma Aldrich) (100 µg/mL): 0.0100 g enrofloxacin, florfenicol, tylosin were dissolved in 5.0 mL methanol (96 %) and diluted to the final volume of 100 mL by distilled water (D.W). Working solutions were prepared by diluting the standard solution with 5.0% methanol.

3. Results and discussion

3.1. Successive Ratio Derivative Method

For determination of enrofloxacin, the first derivative of different concentrations of stored spectra of enrofloxacin divided by the standard spectrum of 10 µg/ mL of florfenicol were recorded using $\Delta\lambda$= 2.0 nm to obtain the first ratio spectra. On the other hand, other first derivative spectra of 25 µg/ mL of tylosin divided by 10 µg/mL of florfenicol was taken using $\Delta\lambda$= 2.0 nm to obtain the second ratio spectra. Then the two derivative ratios was

divided to obtain the third ratio spectra which were used for determination of enrofloxacin at 270, and 272 nm in the presence of florfenicol and tylosin . To obtain the mentioned conditions, optimizations of two variables have been carried out.

3.2.1. Method Development and Optimization

Two variables have been considered in this method development and optimization conditions, as follows:

3.2.1.1. Effect of Divisor Concentration

Different concentrations of divisors (1.0 µg/mL, 3.0 µg/mL, 5.0 µg/mL, 10 µg/mL, 20 µg/mL, 30 µg/mL of each of Florfenicol and Tylosin were investigated. The technique selectivity and sensitivity were shown to be significantly affected by adjusting the concentration of the divisors. It was found that 10 µg/mL of **f**lorfenicol leads to best analytical parameters of slope, and correlation coefficient of pre-calibration graphs, therefore it was selected for determination of enrofloxacin as shown in Fig. (1.0).

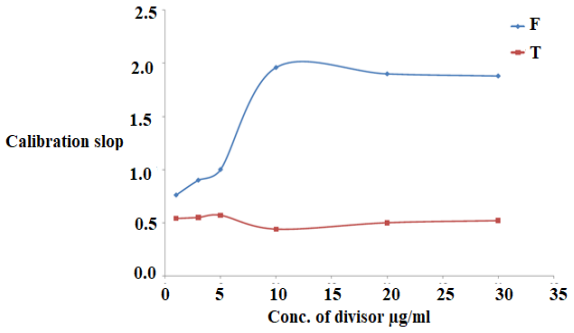

Fig. 1.0 Effect of divisor concentration on sensitivity of enrofloxacin

3.2.1.2. Choice of the Working Wavelength

The calibration curves were constructed using the lowest or greatest of the SR- derivative spectra with respect to wavelengths (16).

The wavelengths selected for estimation of enrofloxacin were 270, and 272 nm, according to their good sensitivity and correlation coefficient. Based on the selected wavelengths, SR-derivative obtained, as shown in Fig. (2.0).

3.2.2. Calibration Graphs

The minimum or maximum peaks of the SR-derivative spectra with respect to wavelengths were used for the construction of calibration graphs by plotting the amplitudes of SR-derivative spectra versus the corresponding concentrations of Enrofloxacin at 270 and 272nm over the concentration range (0.1-25) µg/mL, as shown in Fig. (3.0), with limits of detection 0.024, 0.042 µg/mL, and r^2 equal to 0.9993, 0.9983.

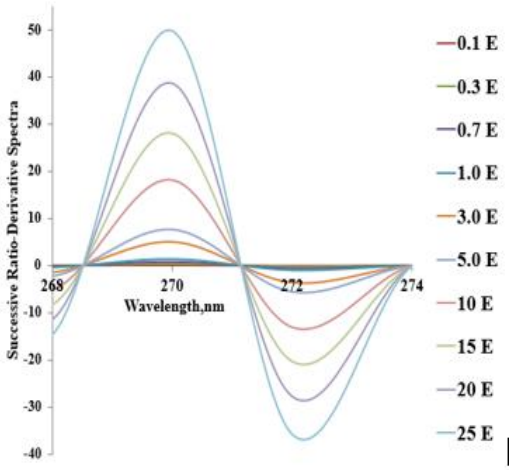

Fig. 2.0: SR-derivative spectra for determination of enrofloxacin

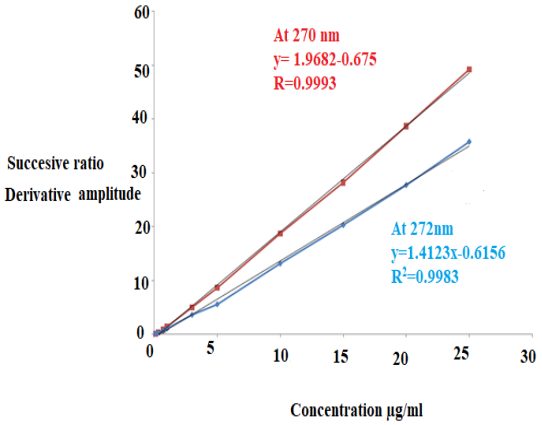

Fig.3.0: Calibration graphs of E using SR- derivative

3.2.3. Precision and Accuracy

Accuracy and precision of sequential ratio-derivative spectrophotometric quantification of enrofloxacin were investigated by calculating the values of RSD and Error for five replicate measurements of three distinct concentrations. Values of RSD% and Error% (as shown in Table 1.0) of low concentrations (0.08 and 0.1) µg/mL are higher than that of high concentrations, which indicate that the precision and accuracy of low concentrations are less.

Table 1.0: Precision and accuracy for determination of enrofloxacin using SR-D method

Wavelength nm	Conc. µg/mL	RSD%	Error %
270	0.1	7.23	4.12
	15	5.46	2.25
	25	1.13	-3.43
272	0.1	6.34	-4.33
	15	4.67	2.12
	25	1.87	-1.19

Table 2.0: T. limit and Error % for some interfering compounds on the determination of enrofloxacin by SR-D method

Interfering compound	Tolerence (T) limit, µg/mL	Error %
Folic acid	155	+4.58
Sodium ion	875	+4.67
Potassium ion	866	+4.56
Magnesium ion	187	-4.64
Ferric ion	888	-4.80
Gentamicin	72	+4.70
Sulfamethoxazole	80	-3.98
Methoprime	83	-4.02
Oxytetracycline	44	+3.88

3.2.4. Interferences Study

The effect of different compounds, including some of meat tissues, and the common used veterinary drugs in chicken diseases, on the determination of ternary mixtures of enrofloxacin with the proposed SR derivative spectra spectrophotometric method were studied. Results showed that the compounds under study did not interfere in the determination of analytes as shown in Table (2.0).

3.2. Treatment of Samples Using Hollow Fiber Membrane

Prepare sample for analysis is normally required to separate and pre-concentrate the analytes. Hollow fiber membrane or liquid phase microextraction aims to improve the selectivity of extraction, and to minimize the volume of organic solvents and glassware to be used (17).

3.3. Physicochemical properties of Target Drugs

The data illustrated in Table 3.0 (calculated with chem office program using classical physics methods), describe some of physicochemical properties of study drugs, which are important in adsorption process and membrane extraction technique, with regards to compare the size of molecule with size of pores of adsorbents, and pores of hollow fiber and to know the polarity and non-polarity of the molecule, which is also important in explanation of how and range of link of the molecule with the adsorbent.

Table 3: Physicochemical properties of target drugs

Analyte	Surface area, Å^2	Polar area, Å^2	Volume, nm^3
E	601.4	117	0.3133
F	545	140.5	0.2716
T	1311.9	289.3	0.8934

The surface entirety of all polar atoms, predominantly O and N, and their connected hydrogen atoms is defined as the polar surface area or topological polar surface area of a molecule. They are useful parameters for predicting the properties of drug transport. If a molecule isn't polar enough, it won't be able to pass through the membrane and return to the surrounding environment. As a result, overall molecular polarity is important: if the material is too polar, the lipid may be unable to pull the molecule out of the aqueous solvent. (18).

3.4. Hollow Fiber Membrane (HFM)

To extract acidic, basic, or polar analyte from fluid arrangement into an acidic (for basic analyte) orbasic (for acidic analyte) acceptor arrangement, a hollow fiber membrane or liquid-liquid microextraction approach is utilized (19).

3.4.1. Characteristics of Hollow Fiber Membrane

Scanning electron microscope was used as a useful tool to survey the morphology and structure of the hollow fiber membrane, Fig. (4.0) shows the channels inside the microporous hollow fiber wall which acts as pathways for target species.

The hollow fiber membrane, as supplied from membrana, (Wuppertal, Germany), is Q3/2 Accrual polypropylene hollow fiber membrane with main properties shown in Table 4.0.

Table 4.0: Main properties of HFM

Matrix type	polypropylene HFM
Pore size	200 nm
Internal diameter	600 nm
Wall thickness	200nm

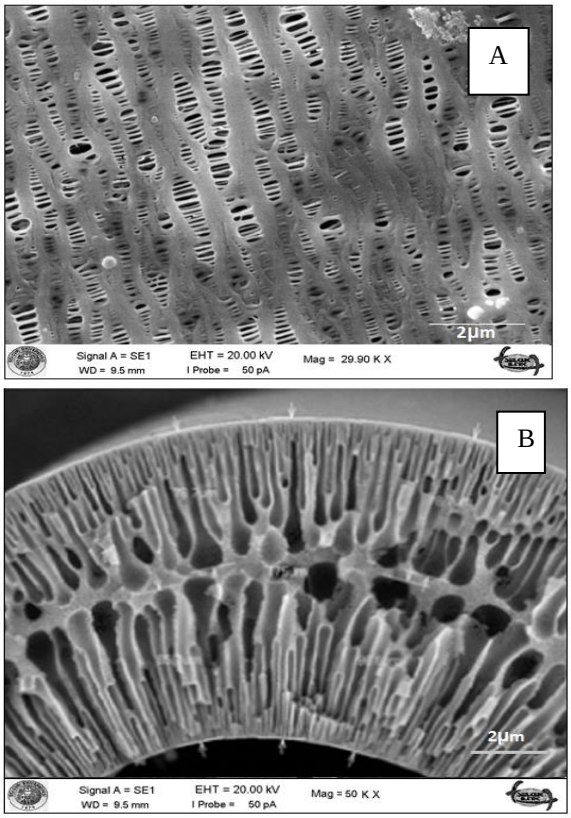

Fig. 4.0: Scanning electron microscope (A) normal image, and (B) cross section

3.4.2. Mechanism of Analytes Transport

The mechanism of enrofloxacin transport (two phase hollow fiber-liquid phase microextraction) through SLM with anionic carrier 2-Ethylhexyl diphenyl phosphate is based on the formation of neutral complex at the donor/membrane interface, between molecules of positively charged analytes and negatively charged carrier immobilized in membrane phase. In next step, the created complex migrates into organic phase and breaks up at the membrane/acceptor interface. Then, instead of analytes, the carrier binds a proton. The driving force of the process is the proton gradient between donor and acceptor(as shown in Fig. 5). The gradient of protons ensures that the transport of target analytes goes only in one direction—from donor to acceptor phase, and high extraction efficiencies can be obtained (20).

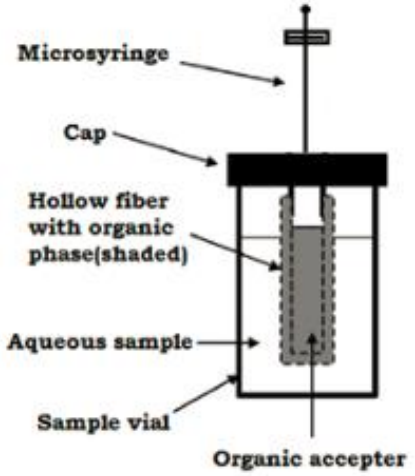

Fig. 5: Principle of two phase hollow fiber-liquid phase microextraction

3.4.3. Extraction Procedure

The extraction and injection procedures were carried out as follows:

A 10 mL test arrangement was exchanged into a 12 mL glass vial containing 1.0 percent sodium chloride and a attractive mixing bar; the vial was set on a attractive stirrer, and the syringe (which was associated to the hollow fiber) was clamped in such a way that the HF piece was totally drenched within the benefactor stage, which contained the pH 5.0 sample. The hollow fiber was taken from the sample solution, its closed end was severed, and the receiving phase was withdrawn into the syringe after the vial was covered and agitated for 80 minutes (15). A 3.0 mL of 5.0 % methanol was added to the receiving phase (24 µL). The concentration of the analytes were found in the solution using SR-derivative spectra at 270nm for determination of enrofloxacin. The extraction procedure is shown in Fig. 6.0

Fig. 6: Extraction process using hollow fiber membrane

3.4.4. Optimization of the Method

The purpose of this study was to see if the hollow fFiber liquid-phase Microextraction could be used to extract the target analytes , The enrichment factor (which is characterized as the proportion of the concentration of analytes within the acceptor (CA) after extraction divided by its concentration within the test some time recently extraction (CS), was investigated and plotted as a work of enrichment factor (Ef). CA/Cs = enrichment factor (20).

3.4.4.1. Choice of liquid Membrane Composition

The choice of organic solvent, for preparation of membrane phase, is important factor because it determines the stability of supported liquid membrane system.(21) It has been proven, that proper organic solvent should possess low water solubility and viscosity since it provides large diffusion coefficients and high fluxes of analytes through the hydrophobic liquid membrane (20), according to these properties different solvents including n-octanol, n-octane, isobutyl methyl ketone (IBMK), and decanol were tested as supported liquid membrane for extraction of the target analytesExperiments with liquid membranes made of pure solvents were used to confirm the necessity of carrier application. There was no analyte transfer from the donor phase to the acceptor solution. The addition of an anionic carrier to the membrane phase, on the other hand, greatly increased the enrichment of the examined analytes. Based on literature survey as well as according to the carriers types, 2-ethylhexyl diphenyl phosphate that exhibits acidic character was elected for extraction of target analytes(22, 23). The presence of an anionic carrier in the liquid membrane in the devised system allows Enrofloxacin, Florfenicol, and Tylosin cations to be transported from the donor to the acceptor phase by forming a neutral complex between the carrier and the investigated analytes. The results in Fig. (7) show that 2-ethylhexyl diphenyl phosphate in isobutyl methyl ketone gave better enrichment factor than the other solvents. The maximum values of enrichment factor are found for solution with 30% (v/v) concentration of 2-ethylhexyl diphenyl phosphate in isobutyl methyl ketone gave. This is owing to the fact that analyte transport is more rapidly due to the increased amount of carrier molecules involved in the cation exchange process. However increasing in carriers concentration, are not suggested since they raise the viscosity of the membrane phase and do not improve the enrichment factor (20).

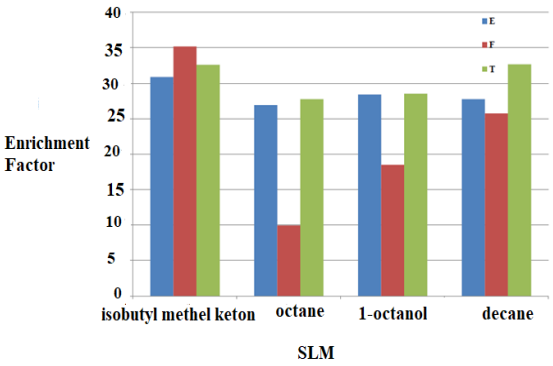

Fig. 7: Effect of supported liquid membrane on enrichment factor of enrofloxacin, florfenicol, and tylosin

3.4.4.2. Effect of the pH of Donor and Acceptor Phases

The pKa's of the studied antibiotics are pKa_1= 5.88-6.06, and pKa_2= 7.7-7.74 for Enrofloxacin(24), 9.03, 7.7 for Florfenicol(25), and Tylosin(26) respectively. These values of pKa of target analytes means that these compounds in a mixture are obtainable in aqueous solution as permanently charged molecules in the whole pH range. There is no pH value at which the analytes under investigation can be present in solution as uncharged molecules capable of diffusing through a hydrophobic organic liquid membrane. As a result, the use of a carrier ethylhexyl diphenyl phosphate was required to insert the ion pair into the membrane phase, allowing for the extraction of multicharged compounds via supported liquid membrane.

To effectively extract to the supported liquid membrane. and produce a high enrichment factor for the basic chemical, the sample solution should be positively charged. The effect of donor phase pH in the range of 3.0-8.0 has been investigated. At 500 rpm, the extractions were completed in 15 minutes. The findings reveal that the enrichment factor of ternary combinations of target analytes increases with pH and peaks at pH 5, as a result of protonation of the molecules, the extraction efficiency reaches 82.13, 91.75, and 97.2%, while the enrichment factor reaches 34.22, 38.22, 33 for Enrofloxacin, Florfenicol, and Tylosin, respectively. Fig. (8.0 A) shows the dependence of studied analytes enrichment factor on the pH of donor phase.

However, further rise of samples pH (>5), decreases the values of enrichment factor. This decrease coincides with a decrease in the amount of cationic molecules in the donor solution. On the other

hand, a pH of less than 5 is not recommended since the driving power (proton gradient) for supported liquid membrane transport is greatly reduced (20).

The presence of a proton gradient between the acceptor and donor phases is a critical parameter for obtaining a high mass transfer. The proton gradient is the driving force behind the anionic carrier ethylhexyl diphenyl phosphate process. Different concentrations of HCl are used in the acceptor phase (0.01, 0.03, 0.05, 0.1)M was tested. At 0.03 M hydrochloric acid, the enrichment factor reaches the maximum, which attributed to complete extraction of basic target analytes to the acidic acceptor phase, as shown in Fig. (8.0B).

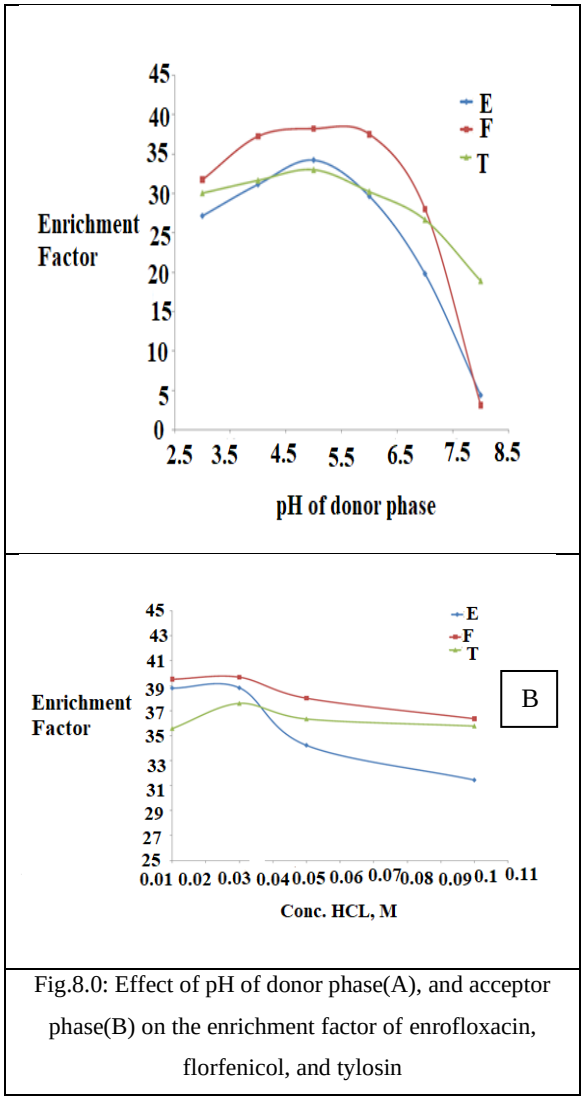

Fig.8.0: Effect of pH of donor phase(A), and acceptor phase(B) on the enrichment factor of enrofloxacin, florfenicol, and tylosin

3.5. *Extraction Time*

Watery standard arrangements were shaken for shifted extraction times extending from 20 to 120 minutes at a blending rate of 500 rpm to explore the impact of the extraction time. The enrichment factor of the extraction expanded with the extraction time up to 80 minutes, as shown in Fig. (9.0), after which there was no increase, indicating that equilibrium had been reached. However, during prolonged extraction time, a slight decrease was observed, most likely due to solvent losses (27).

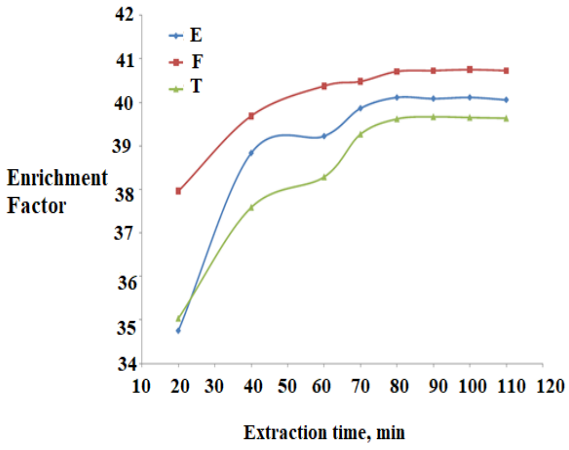

Fig.9.0 : Effect of extraction time on enrichment factor of enrofloxacin, florfenicol, and tylosin

3.6. *Effect of Ionic Strength*

In common, dependent on the nature of the target analyte, including salt to the test arrangement can decrease their solvency and, as a result, increment their hydrophobicity; usually due to the salting-out impact, in which fewer water particles are accessible for dissolving the analytes atoms, shaping hydration circles around the salt particles (14, 28). In our case, the impact of distinctive concentrations (0.5, 1.0, 1.5, 2.0, 2.5, and 3.0%) of Nacl on the enrichment factor of the analytes was evaluated. Based on the results shown in Fig. (10), it could be concluded that the enrichment factor increased with NaCl concentration of 1.0%. Decreasing of the enrichment factor in the higher concentration of sodium chloride is attributed to the electrostatic interaction of salt molecules with polar groups of the analytes (29). Therefore, a NaCl concentration of 1.0% in the donor phase solution was chosen in the present work.

3.7. *Accuracy and Precision of the Extraction Method*

Under optimized conditions, the precision and accuracy of the extraction method for determination of E in the presence of florfenicol and tylosin were checked, depending on the values of RSD % and Error %, which were 1.4-6.1% and 1.4-3.62% respectively.

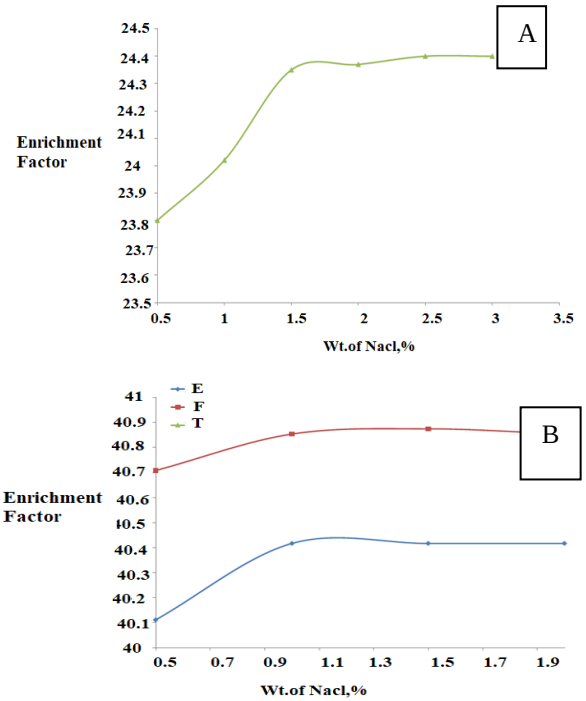

Fig. 10: Effect of ionic strength on enrichment factor of enrofloxacin, florfenicol(A), and tylosin(B)

3.8. Application of the Method

The created approach of Hollow-fiber liquid-phase microextraction successive derivative ratio spectra technique was employed with the aid of standard addition technique to evaluate the applicability of the suggested Hollow-fiber liquid-phase microextraction successive derivative spectra technique for determination of Enrofloxacin in the presence of florfenicol and tylosin in chicken tissues. Muscles (thigh, wing), kidney, and liver was sampled from three different weights of chicken: 2.3, 3.1, and 4.0 kg. The results demonstrate that enrofloxacin was not discovered in the samples. The recovery range is 95.33- 99.66 % .Table (5.0) summarized the results of application and recovery % study.

Table (5.0): Determination of enrofloxacin in the presence of florfrenicol and tylosin in chicken samples with its recovery % using HF-LPME-SD-ratio spectra method.

Sample wt. (kg)	Target Tissue	Found (µg/g)	Recovery%
2.3	Muscle	14.30	95.33
	Liver	14.71	98.06
	Kidney	14.73	98.20
3.1	Muscle	14.65	97.67
	Liver	14.73	98.20
	Kidney	14.85	99.00
4.0	Muscle	14.66	97.73
	Liver	14.83	98.86
	Kidney	14.95	99.66

4. Conclusion

Enrofloxacin, was determined in presence of florfenicol and tylosin using successive derivative spectrophotometry. The direct UV-absorbance measurements or zero-order are subjected to interference from co-formulated matrices and spectral overlapping, As a result, derivative spectrophotometry is a useful analytical technique for interpreting quantitative information from spectra of mixtures containing two or more components with overlapped UV spectra, eliminating matrix interference, improving sensitivity and specificity in mixtures analysis, and so on. The analyses of samples were performed with successive derivative spectra-using hallow fiber was used as LPME.

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