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Research Article

Square Wave and Differential Pulse Voltammetric Determination of Meloxicam in Pharmaceutical Formulations

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ABSTRACT:
Two new voltammetric methods (square wave (SWV) and differential pulse (DPV)) were developed and validated for the direct determination of meloxicam (MX) in pharmaceutical formulations (PFs) without any pre-processes steps. The anodic peaks were obtained in buffer (pH 4.85) on glassy carbon electrode (GCE). The both voltammetric methods were linear at concentration range of 10-90 μg/mL in PF. The validation of methods for MX in PF were determined by establishing specificity, linearity, sensitivity, precision, accuracy, recovery and ruggedness. The recovery results of MX in PFs were found as % 98.5 for SWV and % 98.7 for DPV, respectively. The both developed voltammetric methods were successfully applied for the determination of MX in PFs named Melox, Melcam and Zeloxim. The endogenous substances found in PF were not create electroactive interferences for determine MX. The obtained analysis results of the PFs containing MX by voltammetric methods were compared by using the student t-test with the claimed values and no found statistically differences. It is claimed that new voltammetric methods can be used routinely MX analysis in PFs.

Keywords: Differential pulse voltammetry, meloxicam, pharmaceutical, square wave voltammetry

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1. INTRODUCTION

One of the class of drugs approved by the FDA for mostly prescription as antipyretic, anti-inflammatory, and analgesic agents is non-steroidal anti-inflammatory drugs (NSAIDs) [1]. NSAIDs are used in the treatment of muscle pain, different arthritis, dysmenorrhea, migraines, pyrexia, gout and acute trauma [2]. Also, they are used for treatment of various cancers such as breast, colon, prostate, gastric and ovarian, cardiovascular diseases such as myocardial infarction, stroke and thrombosis, diabetes and central nervous system diseases such as Alzheimer and Parkinson's [3].

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Meloxicam (MX), is a NSAIDs that has sturucture the 4-hydroxy-2-methyl-*N*-(5-methyl-2-thiazolyl)-2H-1,2 benzothiazine-3-carboxamide-1,1-dioxide (**Fig. 1**) and has been approved by the US-FDA in 2000 year. It is used in the treatment of acute and chronic pain and inflammation, as well as to reduce swelling, joint diseases, rheumatoid arthritis and osteoarthritis (4).

Figure 1. Chemical structure of MX

According to current literature, several quantitative analytical methods have been developed for the MX in bulk drug and pharmaceutical formulations (PFs) which are colorimetric [5] UV and derivative spectrophotometric [6-9], spectrofluorometric [7, 9, 10], capillary zone electrophoresis [11] and high-performance liquid chromatography (HPLC) with UV detector [12], or with diode array detector [13]. There are some disadvantages of this commonly used methods. Spectrophotometric methods have low sensitivity. In chromatographic methods total run time is relatively high and high-cost and they require derivatization or extractions.

Thus, quite sensitive, faster, simpler, and cheaper electrochemical methods is being considered as an alternative method. Firstly, reduction of MX was used for its determination by polarography [14, 15], after that cathodic adsorptive stripping square wave voltammetric (SWV) [16] and cathodic adsorptive striping differential pulse voltammetric (DPV) [17] was used for determine of MX. According to this studies, reduction of the double bond in the enol form is the first step, and the reduction of the carbonyl group of the keto form is the second reduction step. The oxidative voltammetric behavior of MX at a carbon paste electrode was investigated by Radi et al [18] with linear scan voltammetry. Developing new validated quantitative method is a very important step in determining the amount of any PFs. Electroanalytical techniques were conventionally preferred for the quantitation of several pharmaceuticals with the advantages that there are instances no necessity for derivatization process and that these methods are unsusceptible from the matrix than other analytical common techniques. In addition to this, electrochemical application includes the determination of mechanism of electrode. Properties of redox of pharmaceuticals can provide insights into their metabolic properties or their in vivo redox process or pharmacologic activity. Although the analytical significance of the electrochemical behavior and oxidation of MX, there were no study that

investigate the square wave voltammetry (SWV) and differential pulse voltammetry (DPV) study of the electrochemical oxidation of MX in PFs.
The main goal of this study was develop and validate a unique voltammetric methods for the direct determination of MX in PFs without any pre-processing extraction step. This paper proposes a fully validated, simple, rapid, selective and sensitive procedures for the determination of MX employing SWV and DPV methods at the GCE, and also a determination the oxidation mechanism of MX by using cyclic, SWV and DPV methods.

2. MATERIAL AND METHODS

2.1. Materials

MX was purchased from Sigma-Aldrich (Germany). Melox, Melcam and Zeloxim tablets were buy from the pharmacy (Erzurum, Turkey). H_2SO_4, Britton-Robinson Buffer (pH 11/pH 8/pH 2), H_3PO_4, CH_3COOH and NaOH were purchased from Sigma-Aldrich (Germany), and double de-ionized water (Milli-Q water, (Barnstead, EASYpure RF, US) and all other chemicals were analytical grade. PF samples (Melox, Melcam and Zeloxim) have been purchased in Erzurum/Turkey.

2.2. Stock and Reagents

Supporting electrolytes solution (SEs) was prepared with 0.2 M phosphate buffer (pH:2-12), 0.2 M $CH_3COOH\backslash CH_3COONa$ buffer (pH:3.5/5.7), 0.5 M H_2SO_4, 0.04 M Britton-Robinson buffer (pH:2-12). MX stock solution (100 µg/mL) was prepared in SEs. Calibration working and quality control (QC) solutions were prepared by diluting the stock solution with SEs.

2.3. Instruments and Method Conditions

Both SWV and DPV analysis were performed via Gamry Potentiostat, the Interface 1000, three electrode Teflon cell, using a BAS 100 W electrochemical analyzer. In all analysis, an Ag/AgCl, 3M NaCl electrode used as the reference electrode, and a platinum wire was served as the counter electrode. A glassy carbon electrode (Φ: 3 mm) were selected as working electrodes during the electrochemical oxidation of MX. The working electrodes was polished before each analysis with polished alumina prepared from 0.01 µm aluminum oxide, and then washed with distilled water. Method conditions were determined as 15 Hz frequency, 25 mV, 4mV potential step for SWV pulse amplitude and 50 ms pulse width, 50 mV, 20 mV/s scan rate for DPV pulse amplitude. pH measurements were carried out with Model 538 pH meter (WTW, Austria. All experiments were carried out at room temperature.

2.4. Preparation of Pharmaceutical Formulations

10 PFs for each formulation (Melox, Melcam and Zeloxim) were weighed and powdered. The amount from one tablet MX contents were transferred into calibrated flasks. They were added the SEs containing $CH_3COOH\backslash CH_3COONa$ buffer (0.2 M, pH 4.85)/NaOH (0.2 M) (v:v, 1:1) and then filled to volume with same solution. The obtained drug solutions were sonicated for 10 min, cooled to

room temperature, filtered (Whatman filter No:42) and desired concentrations for measurements were diluted.

3. RESULTS AND DISCUSSION

3.1. Electrochemical behavior of MX

The voltametric behavior of MX was determined at the GCE in SEs containing CH3COOH\CH3COONa buffer (0.2 M, pH 4.85)/NaOH (0.2 M) (v:v, 1:1) with cyclic voltammetry. Cyclic voltammetric curves data were obtained on GCE in SEs solution, containing 50 µg/mL MX at 0.1 V/s scan rate, as shown in **Fig. 2**.

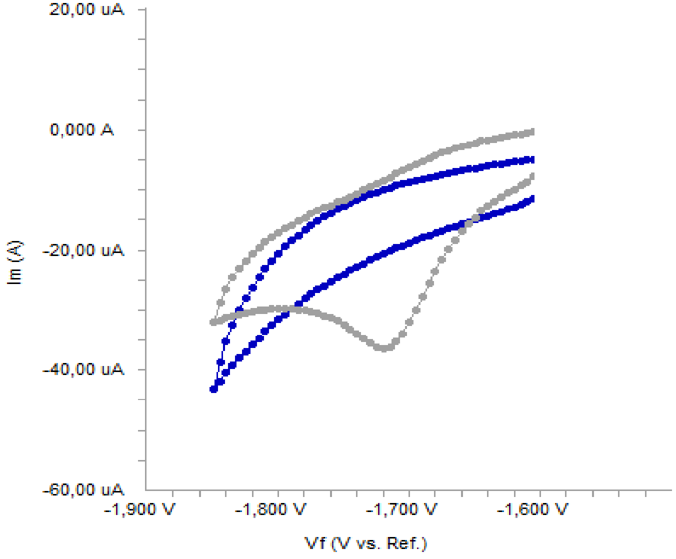

Figure 2. CVs of GCE in SEs (CH3COOH\CH3COONa buffer (0.2 M, pH 4.85)/NaOH (0.2 M) (v:v, 1:1) containing 50 µg/mL MX

An oxidation peak with -1.72 V potentials was observed in the cathodic sweep. But, no reduction peaks were observed in the reverse potential scan. This showed that the electrode reactions were irreversible. So, scan rate in the between 0.01 and 1 V/s on oxidation peak currents and peak potentials were determined in SEs (CH3COOH\CH3COONa buffer (0.2 M, pH 4.85)/NaOH (0.2 M) (v:v, 1:1) containing 50 µg/mL MX. The peak current varies linearly with the scan rate, indicating the adsorption controlled process. Besides, the plots of log (peak currents) versus log (scan rates) for 50 µg/mL MX was found as 0.4947, which this value indicates behaving as ideal diffusion-controlled electrode process (theoretical value: 0.5) [19].

The obtained results confirm that the redox species remain freely from the solution and there is no precipitation on the electrode surface. This behavior is caused from the solubility of the intermediate species or poor adherence of products on the electrode surface. The relationship between the peak potential and scan rate is described by the following equation:

$$E_{pa} = E^{0'} + RT/[(1-\alpha)n_a F][0.78 + \ln(D^{1/2}k_s^{-1}) - 0.5\ln RT/[(1-\alpha)n_a F]] + RT/[(1-\alpha)n_a F]/2\ln v$$

(α: transfer coefficient, n_a: number of electrons transferred). The plots of the peak potentials versus the scan rate for oxidation peak showed linearity according to this equation. The αn_a was found as 0.52 for peak. Also, this value obtained indicate the total irreversibility of the electron transfer processes. This result show that the chemical step is a fast following reaction coupled to a charge transfer.

3.2. Validation of the SWV and DPV methods

The validation was carried out according to ICH Q2B recommendations with specificity, linearity, precision, accuracy, sensitivity (LOD and LOQ), recovery, ruggedness validation parameters [20].

Specificity: Excipients (magnesium stearate, corn starch, sodium laurylsulfate, lactose, polyethyleneglycol, carboxymethylcellulose, titanium dioxide, hydroxypropylmethylcellulose and talc) were spiked to the PF to determine recovery, according to the manufacturer's batch formulas for 15 mg MX per PF. The mean percentage recovery of 25 µg/mL MX showed no significant interference. So, the SWV and DPV methods are specific in that can analysis MX in the presence of excipients.

Linearity: The linearity of SWV and DPV methods were shown by calibration curves obtained by plotting peak current responses of MX versus MX concentration (10, 20, 40, 60, 70, 80 and 90 µg/mL). The obtained SWV and DPV voltammograms for different concentration of MX were shown in **Fig. 3 and 4**, respectively. The linear regression equation for each method was calculated by least squares regression analysis. In addition, standard deviation of intercept (Sa) and slope (Sb) of regression lines from these six linear regression equations were calculated. For SWV and DPV methods, the correlation coefficient was found ad 0.998 and 0.996 respectively. The statistical values are summarized in **Table 1**.

Accuracy and Precision: For each methods, both precision and accuracy were determined with intra-day (6 times per day) and inter-day (6 times once daily for 6 days) analysis of QC samples (20, 40 and 80 µg/mL). The precision of SWV and DPV methods was given by the percent relative standard deviation (RSD %) which calculated as ≤ 2.72 % to ≤ 3.06 %, respectively. The accuracy of method was given by percent Relative Error (RE %) and found as ±2.39 % and ±2.19 % for SWV and DPV methods, respectively. The obtained data are shown in **Table 1.**

Table 1. The statistical values obtained by SWP and DPV methods for determination of MX

Parameters	SWV	DPV
Measured potential (V)	-1.720	-1.720
Linearity (µg/mL)	10-90	10-90
Slope	0.142	0.043
Intercept	10.14	3.944
R	0.998	0.996
S_a	3.453	0.478
S_b	0.524	0.045
LOQ (µg/mL)	1.50	1.50
LOD (µg/mL)	0.50	0.50
Precision (RSD%)	2.72	3.06
Accuracy (% relative error)	-2.39	2.19
Reproducibility of peak current (RSD%)[a]	2.24	3.19
Reproducibility of peak potential (RSD%)[a]	2.33	3.28
Repeatability of peak current (RSD%)[a]	1.48	1.98
Repeatability of peak potential (RSD%) [a]	1.02	1.93

[a]Average of six replicate determinations, RSD: Relative standard deviation, LOD: Limit of detection, LOQ: Limit of quantification S_b: Standard deviation of slope of regression line, S_a: Standard deviation of intercept of regression line R: Coefficient of correlation

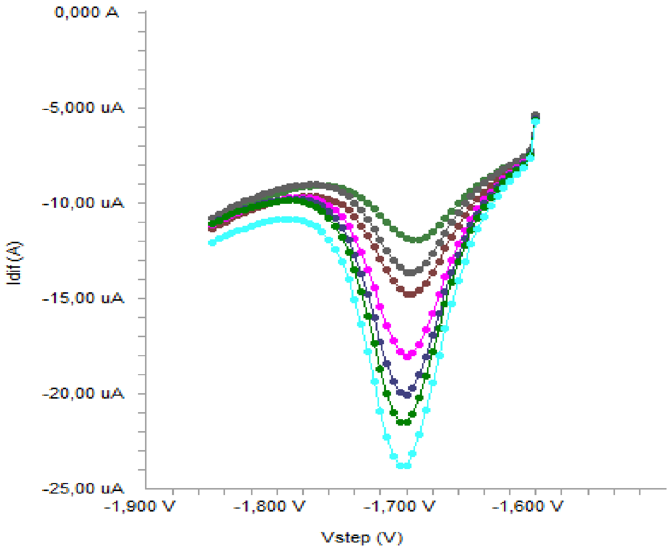

Figure 3. SWV voltammograms for different concentration of MX (10, 20, 40, 60, 70, 80 and 90 µg/mL) in SEs (CH3COOH\CH3COONa buffer (0.2 M, pH 4.85)/NaOH (0.2 M) (v:v, 1:1).

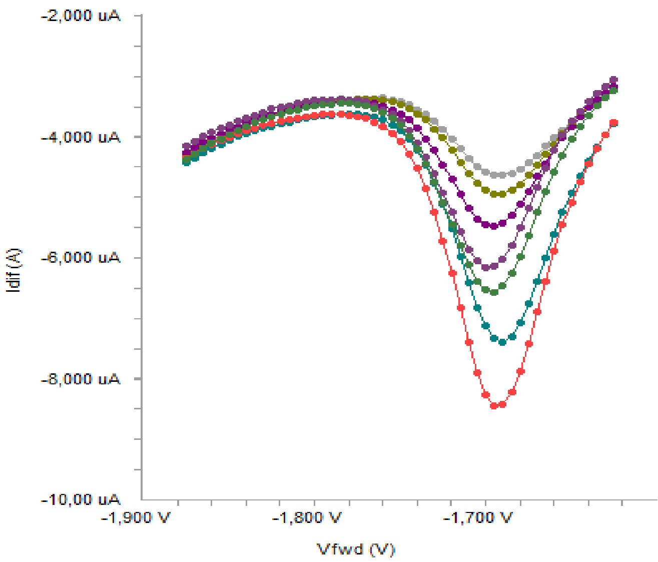

Figure 4. DPV voltammograms for different concentration of MX (10, 20, 40, 60, 70, 80 and 90 µg/mL) in SEs (CH3COOH\CH3COONa buffer (0.2 M, pH 4.85)/NaOH (0.2 M) (v:v, 1:1).

Limits of detection (LOD) and Limits of quantification (LOQ): LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively (σ: standard deviation of *y*-intercepts and S: slope of the calibration curve). The LOD and LOQ were found as 0.50 µg/mL and 1.500 µg/mL for both methods (**Table 1**). These values are sufficient for MX analysis in the PFs.

Recovery: The recovery (R %) were determined by spiking of QC samples to 25 µg/mL MX with necessary dilutions in PFs. The R% of both SWV and DPV methods were found as ≥ 98.5 % and ≥ 98.7 %, indicating good accuracy.

Ruggedness: The SWV and DPV determination of MX were carried out by a different analyst in same instrument with the same standard. The results showed no statistical differences between different operators suggesting that the developed method was rugged.

Determination of MX in PFs with SWV and DPV Methods and Their Comparison with Reference methods in Literature

The developed and validated SWV and DPV methods were applied to analyze the commercially available PFs of Melox, Melcam and Zeloxim tablets Ten replicates determination was made. The obtained results from both methods shown the good recoveries and high reliability for MX in each PFs and was in close agreement with the claimed value. They were statistically compared with each other by using *student-t test* It was not found significant difference between SWV and DPV methods. ($p<0.05$) (**Table 2**). In addition, the obtained results for both methods were statistically compared with the results obtained from reference UV spectrophotometric method (6) and capillary zone electrophoresis by using *F-test* (11) and there was non-significant difference between developed SWV and DPV methods with reference methods ($p<0.05$) (**Table 2**). However the obtained results from the both SWV and DPV methods indicate that these methods are more

accurate and precise for the determination of MX in PFs samples. The voltammetric methods owing to the low cost, high sensitivity, short analysis time and simplicity are important methods for PF [21].

Table 2. Determination of MX with proposed method and comparison with reported methods

Parameters	SWV	DPV	Reported method (Garcia et al. 2000)	Reported method (Nemutlu and Kir 2003)
Mean (recovery %)	99.8	100.1	99.8	100.9
SD	0.634	1.344	0.12	0.04
% RSD	0.635	1.343	1.54	0.53
Variance	0.402	1.806	-	-
t-test (2.228)[a]	0.921		-	-
F- test (5.1)[a]	4.05		-	-

RSD: Relative standard deviation, SD: Standard deviation of six replicate determinations, , [a]Theoretical values, Theoretical values at $p \leq 0.05$, Ho hypothesis: no statistically significant difference exists between four methods, $F_t > F_c$: Ho hypothesis is accepted ($P > 0.05$)

4. CONCLUSIONS

The SWV and DPV methods was developed, validated and successfully applied to the determine of MX in PFs. SWV and DPV methods was rapid and effective electroanalytical methods with well-established advantages, including good discrimination against low detection limits and background current. And the techniques are requiring less than 1 min to sample run time. So, the developed methods can be effectively used without pretreatment for routine analysis of MX in PF

Conflict of Interest

Author has no personal financial or non-financial interests.

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Research Article

The Effect of Fisetin on KATO-III cell proliferation and invasion

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ABSTRACT:
Fisetin is a natural chemical that widely found in different plants. The aim of this study is to investigate the role of fisetin on proliferation and invasion on KATO-III human gastric carcinoma cell. KATO-III cells were seeded into the plates and 24h after dose-dependent effects of fisetin (50 and 100µM) were tested on cultured KATO-III cells. Real-time cell proliferation impedance analysis was performed up to 72h and the Transwell insert assay was performed. Relative mRNA expressions of MMP2 and MMP9, which are markers of invasion, were measured. Data are presented as fold-change in expression of any group compared to that of control group, using the 2-ΔΔCt method. Statistical comparisons were made using one-way ANOVA followed by Tukey's test. Fisetin exert anti-proliferative and anti-invasion effect on KATO-III cells. According to the cell proliferation impedance results, it was shown that proliferations of all fisetin treated groups were suppressed in dose dependent manner. According to transwell invasion results, 100µM fisetin group showed important inhibitory effects on invasion of KATO-III cells compared to the Control group. Regarding to the mRNA expression results of MMP2 and MMP9, it was shown that 50 and 100µM fisetin treatments significantly decreased these expressions compared to Control group. We conclude that fisetin negatively regulates the invasion and proliferation potentials of KATO-III cells via the MMP9 and MMP2 suppression. Thus, our findings implicate fisetin a potential therapeutic target in KATO-III cells.

Keywords: Fisetin, invasion, KATO-III, proliferation

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1. INTRODUCTION

Cancer is a disease characterized by uncontrolled proliferation of cells and spread to other parts of the body [1]. Cancer cells surround healthy cells and blood vessels to form a new microenvironment. This microenvironment also leads to the formation of new blood vessels that provide oxygen and nutrients necessary for tumor growth, namely angiogenesis [2]. Such uncontrolled division and growth of cancer cells complicates the treatment of the disease.

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Around 14 million people worldwide are affected by cancer in a year, and about 9 million of them die [3] The most common types of cancer are breast cancer in women and prostate cancer in men. The highest death rates are seen in lung cancer patients [4]. This situation in Turkey is similar to the world in general. In our country, approximately 175 thousand people are diagnosed with cancer in a year and breast and lung cancer take the first place [5].
Fisetin (3,7,3′,4′ -tetrahydroxy flavone) is a polyphenolic flavonoid, commonly found in fruits and vegetables such as onions, cucumbers, apples, grapes, persimmon, nuts and strawberries. Fisetin is known to have broad pharmacological effects such as anti-tumor [6], neurotrophic [7], anti-inflammatory [8], antioxidant [9] and antiangiogenic [10].
The aim of this study is to investigate the role of fisetin on proliferation and invasion on KATO-III human gastric carcinoma cell.

2. MATERIAL AND METHOD

Cells were seeded into the plates and 24 h after Dose-dependent effects of fisetin (50 and 100 μM) were tested on cultured KATO-III cells. Relative mRNA expressions of MMP9, which are markers of invasion, were measured. Statistical comparisons were made using one-way ANOVA followed by Tukey's test.
KATO-III cell lines will be cultured in suitable culture media containing 10% serum and 1% penicillin-streptomycin (Life Technologies, USA). Cell lines will be incubated in 370C, 90% humidity and 5% CO2 medium.
Experimental Groups;
1. Control (Cancer Cell lines)
2. Fisetin (at doses of 50, 100 μM)

2.1. Invasion Test

Invasion levels will be performed using Transwell Chambers wells and 24-well cell culture dishes. 750 μl serum medium will be placed in the lower wells and 200 μl (250.000 cells/ml) serum-free medium cells will be planted on the upper side. Appropriate doses of drug applications will be made. Incubate at 37°C for 12 hours. Cell numbers migrating to the membrane will be taken using a Leica invert light microscope.

2.2. Real Time PCR Analysis

RNA Isolation: Tissue samples Total RNA isolation steps in Qiaqube (Qiagen RNA isolation device) using RNeasy Mini Kit (Qiagen) will be performed as recommended by the manufacturer.
Reverse Transcriptase Reaction and cDNA Synthesis: High Capacity cDNA Reverse Transcription Kit will be used to synthesize cDNA from total RNA. Each reaction is carried out with 10 ll RNA and cDNA synthesis will be performed with Veriti 96 Well Thermal Cycler (Applied Biosystem) according to the following temperature values. The amount of cDNA will be determined by nano drop spectrophotometer (EPOCH Take3 Plate, Biotek) and stored at -20˚C.

Quantitative Determination of mRNA Expression by Real Time PCR MMP9 mRNA expression will be quantified using the TaqMan Gene Expression Master Mix kit. Amplification and quantification will be performed on the StepOne Plus Real Time PCR System (Applied Biosystems). B-actin as housekeeping gene will be pipetted as follows and carried out with 40 cycles. Ct values will be taken from the instrument and converted to delta Ct by formula and the findings will be evaluated statistically in IBM SPSS 20.0 package program.

3. RESULTS

Fisetin exert anti-proliferative and anti-invasion effect on KATO-III cells. According to the cell proliferation impedance results, it was shown that proliferations of all fisetin treated groups were suppressed in dose dependent manner. According to transwell invasion results, 100µM fisetin group showed important inhibitory effects on invasion of KATO-III cells compared to the Control group. Regarding to the mRNA expression results of MMP2 and MMP9, it was shown that 50 and 100µM fisetin treatments significantly decreased these expressions compared to Control group. MM9 mRNA expression results are shown figure 1. Real-time cell proliferation impedance analysis results are shown figure 2.

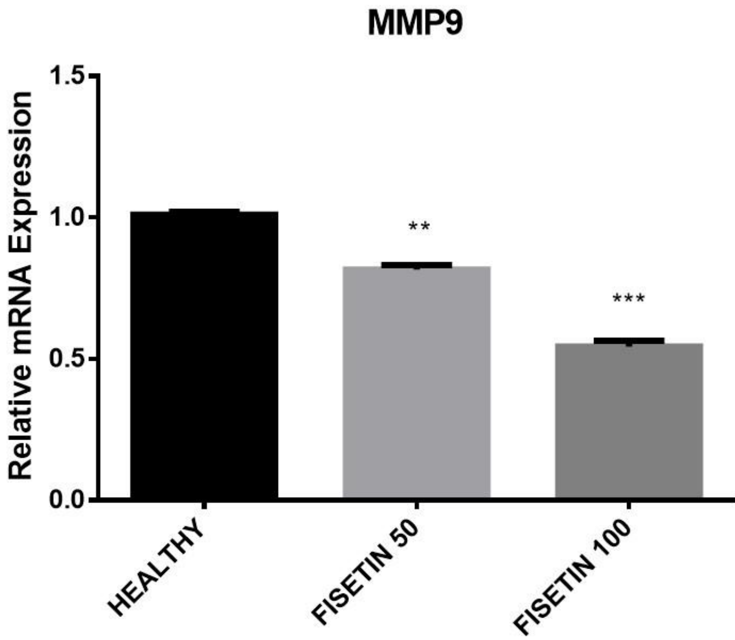

Figure 1. MM9 mRNA Expression Results

Results present as mean±S.D. Data are presented as fold-change in expression of any group compared to that of control group, using the 2-ΔΔCt method. ($p<0,001$).

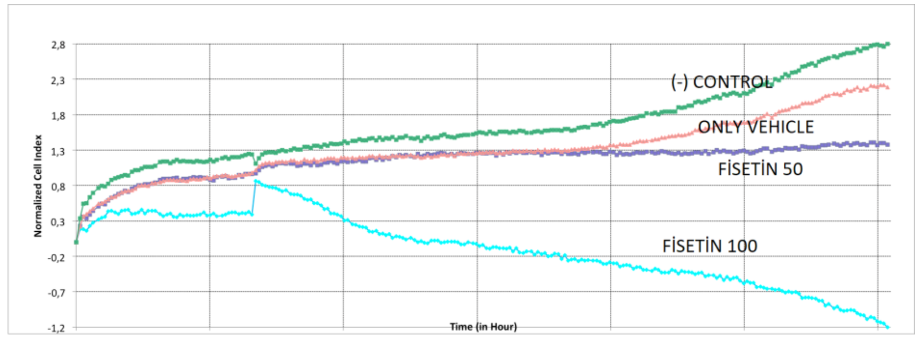

Figure 2. Real-time cell proliferation impedance analysis results

4. CONCLUSION

It was found that fisetin reduces the level of inflammatory cytokines such as TNFα, IL6 and IL-1β [11]. Fisetin inhibited NO, PGE2, IL-1β, and IL-6 production, iNOS and COX-2 expression, NF-κB activation [12]. On the other hand, fisetin has been found to reduce angiogenesis by reducing eNOS, VEGF, EGFR, COX-2 expressions [13].

It has been proven in some previous scientific studies that fisetine decreases the amount of increased MDA and that it decreases the levels of decreased SOD, CAT, GSH and GST to a level close to normal values [14, 15]. Recent findings indicate that administration of fisetin significantly increases antioxidant response element activity and nuclear translocation of Nrf2 [16]. In addition, Fisetin has been shown to inhibit NADH oxidation and ATPase activity [9].

We conclude that fisetin negatively regulates the invasion and proliferation potentials of KATO-III cells via the MMP9 and MMP2 suppression. Thus, our findings implicate fisetin a potential therapeutic target in KATO-III cells.

Acknowledgment

This study was presented at 5th International Eurasian Congress on Natural Nutrition, Healthy Life & Sport, 02-06 October, 2019 - Ankara, Turkey.

Conflict of Interest

Author has no personal financial or non-financial interests.

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Research Article

Determination Of Ciprofloxacin With Zero-, First- And Second-Order Derivative Spectrophotometric Method In Water And Methanol Media

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ABSTRACT:
Ciprofloxacin is a synthetic fluoroquinolone derivative antibiotic used to treat various bacterial infections. Simple, fast and reliable zero-, first- and second-order derivative spectrophotometric methods were developed for determination of ciprofloxacin in two pharmaceutical dosage forms. The solutions of standard and the sample were prepared in methanol and water medium. The quantitative determination of the drug was carried out using the zero-, first- and second-order derivative values measured 270-310 nm (N=6) Calibration graphs constructed at their wavelengths of determination were linear in concentration range of ciprofloxacin using peak to zero 2.00-10.00 µm/mL for zero-, first- and second-order derivative spectrophotometric method. The developed methods were successfully applied for the assay of pharmaceutical dosage forms for two solvent media which do not require any preliminary separation or treatment of the samples. The details of statistical treatment of analytical data are also presented (p>0.05).

Keywords *:* Ciprofloxacin, derivative spectrophotometric method, different media

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1. INTRODUCTION

Ciprofloxacin has a chemical structure of 1-cyclopropyl-6-fluoro-1.4-dihydro-4-oxo-7-(with piperazine) quinolone-3-carboxylic acid (Fig.1.). It is a broad-spectrum antibacterial agent in the structure a fluoroquinolone having against gram positive and gram-negative bacteria. It shows its activity by appearing as bacterial DNA gyrase enzyme Thus bacteria are not resistant to fluoroquinolones through plasmid or R factor mediated mechanisms. and is not vulnerable to degradation by bacterial inactivating mechanisms. Ciprofloxacin is usually used in the infections of gastrointestinal tract, urinary tract, and skin tissues by bacteria [1-8].

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Figure 1. Chemical structure of ciprofloxacin

Various assay methods (HPLC, polarography, adsorptive stripping voltametry and titrimetry) have been reported for determination ciprofloxacin in biologic material and in pharmaceutical preparation [1,4,8-11]. Few spectrophotometric methods have been reported for determination of ciprofloxacin in pharmaceutical preparations. These included complex formation with eosin, paladyum and iron, through the formation of charge transfer complex with chlorinic acid [11-16].

Based on the above reported methods, it was thought necessary to developed simple, fast and accurate spectrophotometric method for determination of ciprofloxacin in pharmaceutical preparations. The aim of this work was to investigate the utility of zero-, first- and second-order derivative spectrophotometry in assay of ciprofloxacin in pharmaceutical preparations without the necessity of sample pre-treatment. In this study, zero-, first- and second-order derivative UV spectrophotometric methods are developed and validated for the determination of ciprofloxacin in two different solvent media (water and methanol). The developed methods were applied to two different commercial preparations as tablet and eye drop. The results obtained from zero-, first- and second-order derivative spectrophotometry to two different solvent media (water and methanol) were compared.

2. MATERIALS AND METHODS

2.1. Instrument

The spectrophotometric measurements were performed on a Thermospectronic double beam UV Vis spectrophotometer, using 1.0 cm quartz cells, connected to Lexmark lazier printer. The spectral bandwidth was 2 nm and wavelength scanning speed was 600 nm min-1. The derivative spectra of test and reference solutions both water and methanol were recorded over the range 270-310 nm with $\Delta\lambda$ = 21.0 nm.

2.2. Materials and Reagents

Reference ciprofloxacin was kindly supplied by the Bilim Drug Company in Turkey. It was tested for purity by controlling its melting point, UV and IR spectrum. The impurity was not found. Cipro tablets, containing 500 mg ciprofloxacin, and Sipragut eyedrop, containing 3.5 mg/mL ciprofloxacin, were obtained from local market in Erzurum-Turkey. All experiments were performed with analytical reagent grade purchased from Merck.

2.3. Standard Solutions of Ciprofloxacin

Stock solutions of ciprofloxacin were prepared at a concentration of 20 μg/mL both methanol and water medium. The solutions kept at room temperature and strict. Stability of ciprofloxacin stock solutions were tested during a period of two days and found to be stable. Working standard solutions were daily prepared by diluting stock solutions at the concentrations of 2, 4, 6, 8 and 10 μg/mL both methanol and water media. Water and methanol were used as blank solution.

2.4. Procedures

A total 5 tablets of ciprofloxacin accurately weighed and powdered. An amount of this powder corresponding to one tablet ciprofloxacin content was weighed and transferred in a 100 mL volumetric flask. 15 mL methanol was added and the flask was sonicated for 5 min. The flask was filled to volume with methanol and the same procedure was made for water. 1 mL of eye drop was transferred to 100 mL volumetric flask, 15 mL methanol was added and the flask was sonicated for 5 min. The flask was filled to volume with methanol and the same procedure was made for water

2.5. Method Validation

The validation of method was carried out by establishing specifity, linearity, recovery values, limits of detection (LOD), limit of quantification, within- and between-day precision and accuracy according to International Conference on Harmonization guidelines (ICH) [17,18] for validation of analytical procedures.

3. RESULTS AND DISCUSSION

3.1. Optimization of Conditions

The solvent, the degree of derivation, the wavelength ranges and N values were chosen in order to optimize the conditions. Optimum results were obtained in measuring wavelength range 270-310 nm, N=6 ($\Delta\lambda$ = 21.0 nm) for zero-, first- and second-order derivative spectrophotometry. UV spectrum of ciprofloxacin gives a single peak at 286 nm. The ^{1}D curve displayed a maximum at 283 and a minimum at 289 nm, while the ^{2}D curve showed a maximum at 280 nm and a minimum at 286 nm both water and methanol medium (Fig.2.). Zero-, first- and second-order derivative UV spectrum of Cipro tablets and Sipragut eyedrop solution both water and methanol medium are shown in Fig. 3 and 4, respectively.

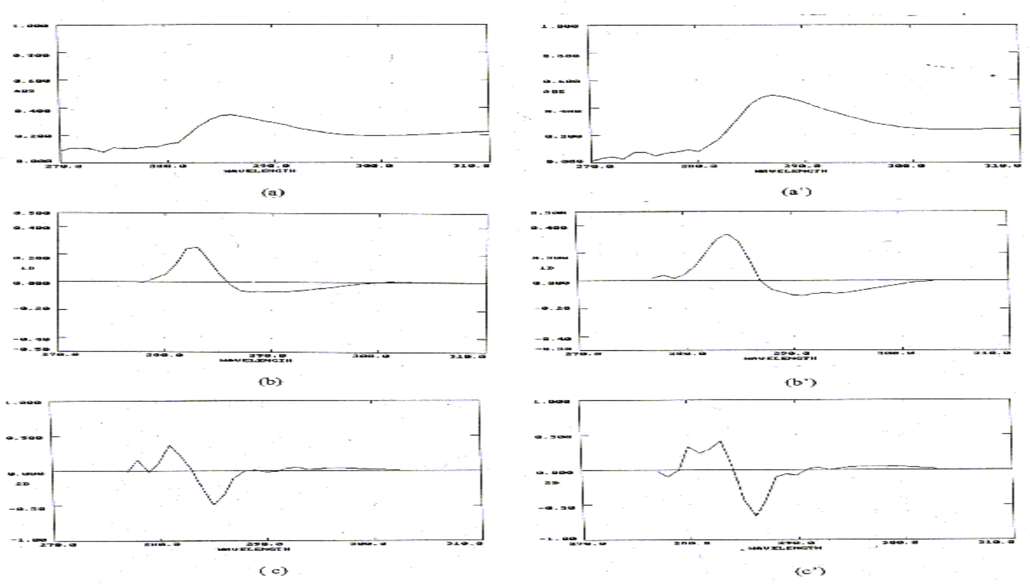

Figure 2. Zero-, First- and Secon-order derivative spectrum of standard ciprofloxacin solution (7μg/mL); in water medium (a,b,c) and in methanol medium (a′,b′,c′)

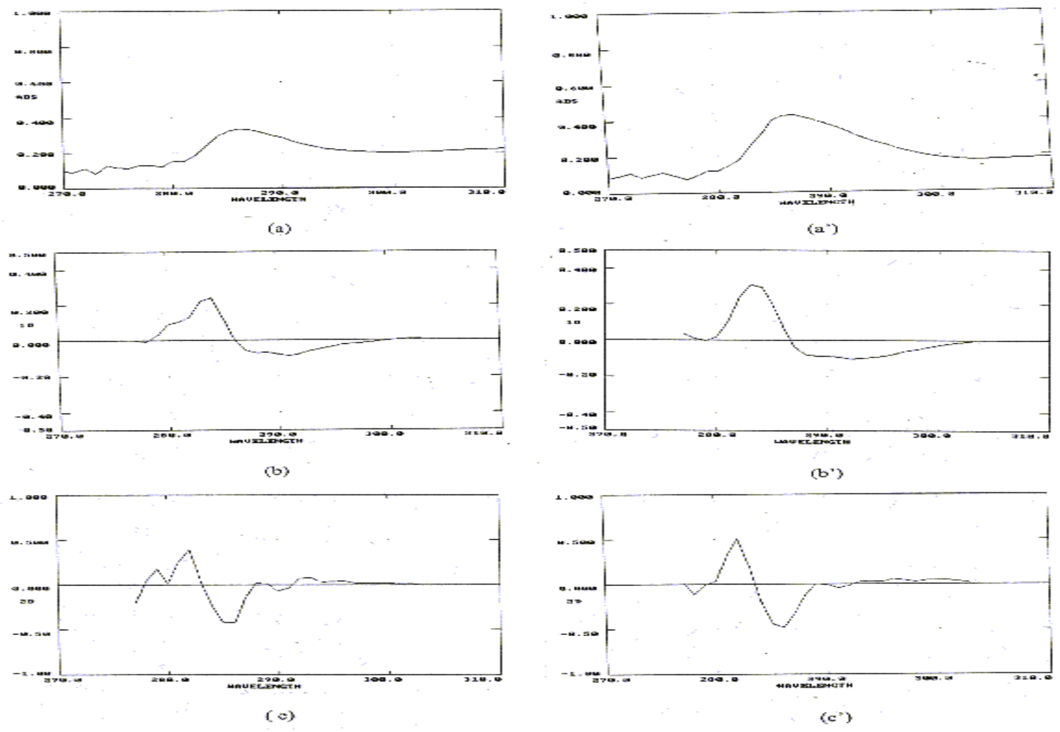

Figure 3. Zero-, First- and Secon-order derivative spectrum of Cipro 500 mg tablet solution (7μg/mL);in water medium (a,b,c) and in methanol medium (a′,b′,c′)

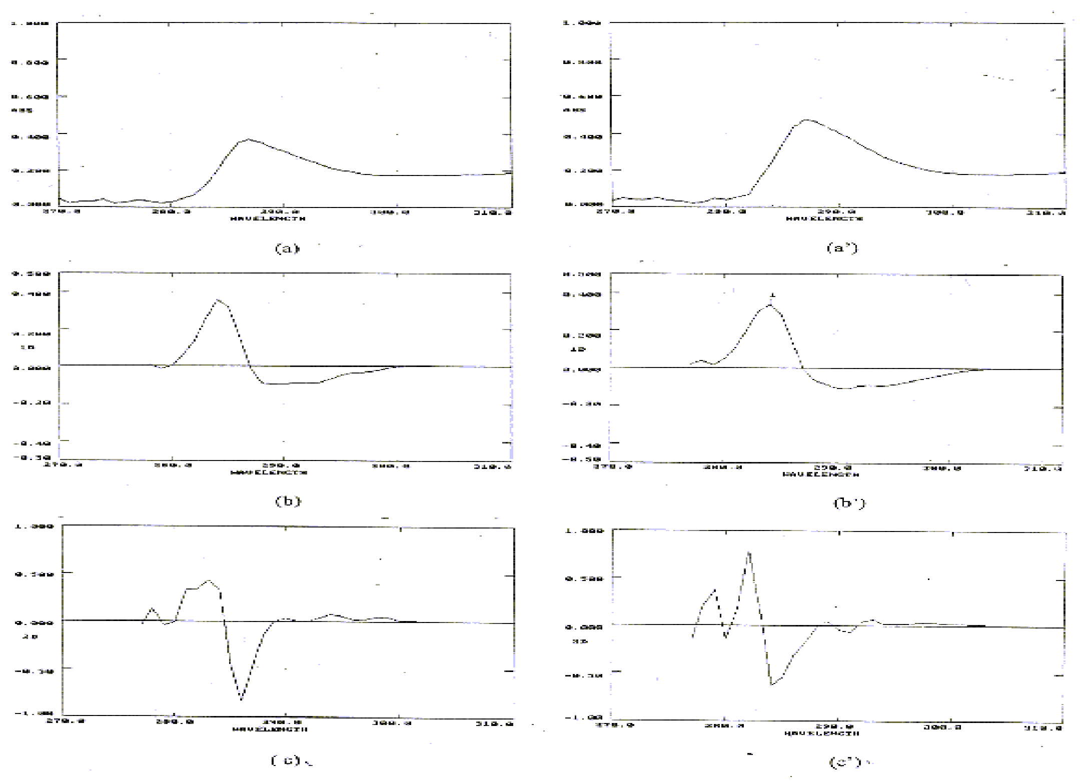

Figure 4. Zero-, First- and Secon-order derivative spectrum of commercial sterilize sipragut eyedrop solution (7μg/mL);in water medium (a,b,c) and in methanol medium (a',b',c')

3.2. Linearity of Calibration Curves

In quantitative analysis, the standard calibration curves for zero-, first- and second-order derivative spectrophotometry of ciprofloxacin in methanol and water medium were constructed by plotting derivative absorbance versus concentration under the experimental conditions described and evaluated by using correlation coefficient. Regression analysis using the method of least-squares was made for the slope, intercept and correlation coefficient values (Table 1 and 2). The regression equations of calibration curves for methanol medium were $y=0.0655x + 0.0289$, $y= 0.0266x + 0.0339$ and $y= 0.4450x + 0.1230$ for zero- first and second-order derivative spectrophotometric methods, respectively. For water medium, the regression equations of calibration curves were $y=0.0442x + 0.0271$, $y= 0.0354x + 0.0565$ and $y= 0.0805x + 0.0091$ for zero- first and second-order derivative spectrophotometric methods, respectively. The linearity ranges were found to be 2-10 μg/mL for both methanol and water media.

The correlation coefficient of standard calibration curves for zero-, first- and second-order derivative spectrophotometry of ciprofloxacin in methanol medium were higher that of ciprofloxacin in water media. Thus it has been found that the methanol solution is better but the correlation coefficients obtained from all of calibration curves of each of these solutions were showed good linearity.

Table 1. Features of the calibration curves zero-first–second-order derivatives of ciprofloxacin in methanol

Features	Zero-order	First-order	Second-order
Regression equation	y=0.0655x +0.02892	y=0.0266x +0.0339	y=0.4450x + 0.1230
RSD%	0.52-4.53	0.58-5.12	0.63-6.48
Correlation coefficent (r)	0.9998	0.9972	0.9945
Linear range (μg/mL)	2-10	2-10	2-10

Table 2: Features of the calibration curves zero-first–second-order derivatives of ciprofloxacin in water

Features	Zero-order	First-order	Second-order
Regression equation	y=0.0442x +0.0271	y=0.0354x +.0565	y=0.0805x +0.0090
%RSD	0.60-5.37	0.65-6.24	0.71-7.25
Correlation coefficent (r)	0.9965	0.9874	0,9884
Linear range (μg/mL)	2-10	2-10	2-10

3.3. Sensitivity

In methanol medium, the limit of quantification (LOQ) for ciprofloxacin was found as 0.5 μg/mL, 0.65 μg/mL and 0.70 μg/mL and the limit of detection (LOD) was found as 0.1 μg/mL (s/n > 2), 0.35 μg/mL and 0.75 μg/mL for zero-, first- and second- order derivative spectrophotometry.

In water medium, the limit of quantification (LOQ) for ciprofloxacin was found as 0.6 μg/mL, 0.85 μg/mL and 0.89 μg/mL and the limit of detection (LOD) was found as 0.2 μg/mL (s/n > 2), 0.55 μg/mL and 0.95 μg/mL for zero-, first- and second-order derivative spectrophotometry. The determinations of different concentration levels were carried out for each drug to test sensitivity, quantitation and reproducibility and of zero-, first- and second- order derivatives values.

3.4. Repeatability

Repeatability is given as inter- and intra-day precision and accuracy where evaluated by analyzing three different concentrations and three different day of ciprofloxacin. The inter-day precision was evaluated by comparing the linear regressions of three standard plots prepared on three different days. Six replicate determinations at three different concentrations (the concentration range 3, 7 and 9 μg/mL for ciprofloxacin in methanol and water) were carried out to test the precision of this method. The experimental results obtained from zero-, first- and second-order derivative spectrum of ciprofloxacin both methanol and water are shown in Table 3, 4 and 5, respectively

The RSD values from ciprofloxacin in methanol and water media, respectively, were found to be 0.51-8,6% and 1.52- 9.5% for zero-order derivative, 1.27-5.72% and 3.21-8.48% for first-order derivative and 2.31-5.43% and 3.12-8.38% for second-order derivative spectrophotometry. These data indicated that the developed methods have a good repeatability.

Table 3. Summary of assay precision data for ciprofloxacin in methanol and water intra-day, inter day by UV-Vis spectrophotometry

		Intra day			Inter-day		
Sample	Concentration (μg/mL)	X	SD	RSD %	X	SD	RSD %
Ciprofloxacin (water)	3	0.1280	0.0550	4.30	0.1300	0,0083	6,41
	5	0.2280	0.0036	1.56	0.2310	0.0190	8.54
	7	0,3150	0.0048	1.52	0.3190	0.0300	9.50
Ciprofloxacin (methanol)	3	0.1940	0.0009	0.51	0.1820	0.0039	2.16
	5	0.3620	0.0054	1.49	0.3120	0.0270	8.60
	7	0,4430	0.0035	0.79	0.4170	0.0148	3.57

SD[a] : Standard deviation of six replicate determinations, RSD: % Relative standard deviation

Table 4. Summary of assay precision data for ciprofloxacin in methanol and water intra-day, inter day by first - order spectrophotometry

		Intra day			Inter-day		
Sample	Concentration (μg/mL)	X	SD	RSD %	X	SD	RSD %
Ciprofloxacin (water)	3	0.1100	0.0093	8.48	0.1190	0.0109	9.21
	5	0.1470	0.0066	4.49	0.1530	0.0049	3.21
	7	0.2250	0.0175	7.80	0.2380	0.0127	5.37
Ciprofloxacin (methanol)	3	0.1470	0.0053	3.65	0.1580	0.0020	1.27
	5	0.2370	0.0089	3.75	0.4510	0.0233	5.17
	7	0.3130	0.0139	4,44	0.2310	0.0132	5.72

SDa : Standard deviation of six replicate determinations, RSD: % Relative standard deviation

Table 5. Summary of assay precision data for ciprofloxacin in methanol and water intra-day, inter day by second-order spectrophotometry

		Intra day			Inter-day		
Sample	Concentration (μg/mL)	X	SD	RSD %	X	SD	RSD %
Ciprofloxacin (water)	3	0.2420	0.0147	6.09	0.2390	0.0074	3.12
	5	0.2930	0.0163	5.56	0.2870	0.0130	4.56
	7	0.4400	0.0368	8.38	0.4350	0.0322	7.42
Ciprofloxacin (methanol)	3	0.2600	0.0141	5.43	0.2710	0.0111	5.12
	5	0.6050	0.0250	4.14	0.7210	0.0166	2.31
	7	0.7990	0.0271	3.39	0.6100	0.0212	3.48

SD[a] : Standard deviation of six replicate determinations, RSD: % Relative standard deviation

4. CONCLUSIONS

An analytical zero-, first- and second-order derivative spectrophotometric methods was developed and validated thoroughly for quantitative determination of ciprofloxacin in two pharmaceutical formulations (tablet and eye drop) in both methanol and water media.

The present method was found to be simple, accurate and reproducible which can be directly and easily applied of the pharmaceutical formulations of Ciprofloxacin.

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Review

Covid-19 and Antiviral Drugs Used In Its Treatment

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ABSTRACT:
The new coronavirus, the seventh family member of β coronaviruses, entered our lives as 2019-nCoV. The first outbreak was seen in December 2019 in Wuhan, China. It has been reported as a zoonotic disease. 2019-nCoV; causes fatal diseases affecting the lungs, heart, liver and the whole body. 2019-nCoV interacts with ACE-2 and infects epithelial cells, initiating endothelial activation, localized inflammation, tissue damage, and dysregulated cytokine release. Antiviral drugs that selectively bind to viral proteases block the proteolytic cleavage of protein precursors necessary to produce infectious, thereby preventing viral replication. Newly designed antiviral drugs act not on viral entry into host cells but instead by blocking one or more steps of virus replication within the cell. Appropriate analytical methods are required to monitor the precise amount, distribution, metabolism, adsorption and elimination of these drugs and their metabolites in biofluids and tissues. These methods are; The preparation of plasma samples includes protein precipitation (PP), solid phase extraction (SPE), liquid-liquid extraction (LLE), or a combination of two or more of these.

Keywords: Antiviral drugs, extraction, Sars-Cov-2

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1.INTRODUCTION

The coronavirus belonging to the Coronaviridae family can be divided into four main genera (α, β, γ and δ) [1]. The new coronavirus belongs to the β coronavirus genus, and 2019-nCoV has entered our lives. After the outbreaks of severe acute respiratory syndrome (SARS)-CoV in 2003 and Middle East respiratory syndrome (MERS)-CoV in 2012, the coronavirus, which was initially reported as 2019-nCoV, then renamed as SARS-; It causes respiratory tract infection, severe pneumonia, and progressive consequences, up to death [2]. Although these coronaviruses have been isolated from different human and animal hosts at different times and places, they all belong to the coronavirus type associated with severe acute respiratory syndrome [3]. The first outbreak appeared in Wuhan, China, in December 2019. It turned out that most of the patients who were first introduced to 2019-nCoV made frequent purchases from the Huanan South Seafood Market in Wuhan, where

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seafood, pheasants, chickens, bats and other animals were sold. Therefore, it has been assumed that the associated disease is a zoonotic disease [4]. According to the data of the World Health Organization, as of March 15, 2020, SARS-CoV-2 spread rapidly with 34 in China, and then 144 countries became infected with the virus [5]. Existing antiviral drugs have been meticulously studied and the most effective treatment method has been tried to be created in order to interfere with the virus spreading so rapidly.

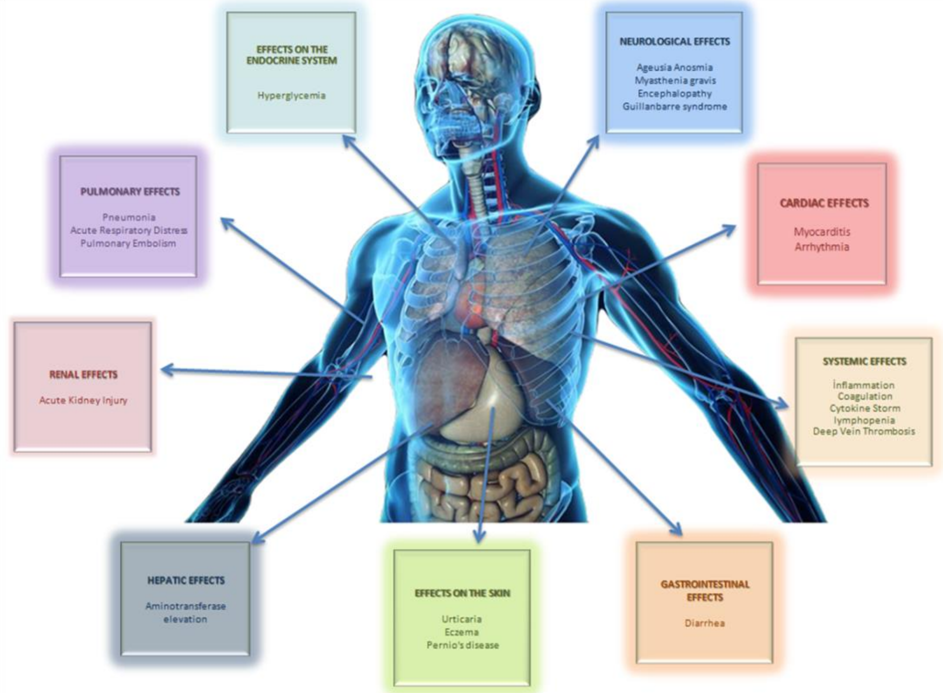

Figure 1. Sars-Cov-2 impacts on health [6]

1..1. Clinical Symptoms of Covid 19

Asymptomatic patients do not have any symptoms, only the test is positive. The patient with mild symptoms has flu-like symptoms. In moderate cases, pneumonia is usually seen, but hypoxemia is usually absent. In severe cases, there is pneumonia accompanied by hypoxemia [7].

1.2. Treatment Mechanisms

Two different methods are used to use existing drugs in the treatment of Sars-Cov-2; The first is the prevention of virus entry into the host cells and the second is the prevention of various steps of virus replication within the cell [8].

1.3. Blocking Viral Entry

SARS-CoV-2 enters the host cell by binding to specific cell surface receptors such as human angiotensin converting enzyme (hACE). It releases its RNA in epithelial cells (ECs). Here it is secreted to replicate to neighboring cells for further infection and spread through the nasal passage to the alveolar region of the lung. It has been subjected to many antiviral tests in both in vitro studies and clinical studies. Most of the antivirals tested are protease inhibitors. Redesigned antivirals; Like ACE2 receptor blockers, it does not affect viral entry into host cells, but acts by blocking

the steps of virus replication within the cell. For example; endocytosis can be inhibited, endosome maturation and release of viral genome can be inhibited. In addition, virus replication, transcription and translation of viral proteins can be inhibited [7].

1.4. Antiviral drugs

Favipiravir; Favipiravir is an antiviral agent used for new strains of influenza that survive more severely from seasonal flu [9]. Favipiravir is a prodrug that can be converted to an active form by intracellular phosphoribosylation and is a selective and potent inhibitor of RNA-dependent RNA polymerase (RdRp) of RNA viruses [10].

Remdesivir; Remdesivir, a monophosphoramidate prodrug, is an adenine nucleotide analogue. Remdesivir has broad-spectrum antiviral activity, including filovirus and coronavirus [11]. It shows its effect by interfering with the viral RNA dependent RNA polymerase (RdRp) enzyme. Thus, it causes a delay in chain termination and stops RNA synthesis and viral replication [12].

Ribavirin; Ribavirin is a guanine analog, it acts by inhibiting viral RNA-dependent RNA polymerase. Its effectiveness against other viruses in the coronavirus family has prompted a re-study of Ribavirin for Sars-Cov-2. However; It has been found to have limited in vitro activity against SARS-CoV-2, requiring an additional drug and high concentrations for an effective treatment [13]. The reproductive toxicity and the hemolytic anemia is the mostserious side effects of Ribavirin [14].

Lopinavir-ritonavir; Lopinavir is a protease inhibitor and an antiviral agent effective against Human Immunodeficiency Virus (HIV) [15]. The lopinavir-ritonavir combination has been studied against the SARS-CoV-2 virus. Potent inhibition of cytochrome (CYP) P450 3A4 combined with protease inhibitors in combination with low-dose ritonavir significantly increased the plasma concentration and efficacy of administered lopinavir [16].

Umifenovir; Umifenovir is a broad spectrum antiviral agent. It has activity against Hepatitis C, Hepatitis B, Ebola Virus, Polio Virus, Lassa Virus.3 It inhibits membrane fusion of the viral envelope by targeting the interaction between viral S-proteins and ACE2 receptors [17].

Oseltamivir; Oseltamivir, a neuraminidase inhibitor; It is an antiviral agent with strong efficacy against influenza A and influenza B viruses [18]. Oseltamivir targets neuraminidase distributed on the surface of influenza virus to inactivate influenza virus [19].

1.5. Methods Used for Extraction of Antiviral Drugs

Existing antiviral drugs have been reworked for the treatment of Covid-19. Most of the therapeutic drug monitoring studies are human plasma; carried out in various biological matrices such as urine, saliva, breast milk, cerebrospinal fluid, sperm plasma, feces, poultry muscle and cell lysates. In the preparation of plasma samples; protein precipitation (PP), liquid-liquid extraction (LLE), solid phase extraction (SPE) methods are more preferred. Protein precipitation is most

preferred as it is more affordable. The organic solvent is added to the plasma, thereby changing the solubility of the proteins in water. This causes crashing. The precipitate is separated by centrifugation. It is preferred to use acetonitrile/methanol mixtures in different ratios as a precipitating agent in the precipitation of antiviral drugs. Also, in some cases, acetonitrile acidified with 0.01% HCl or water adjusted with 8% (v/v) trichloroacetic acid (TCA) or methanol alone has been used [20]. In addition to the above, the QuEChERS method was used to extract antiviral drugs from biofluids and biological tissues. The method involved a solvent extraction with acetonitrile followed by cleavage with magnesium sulfate and sodium chloride. The final step involved a dispersive solid phase extraction (d-SPE) cleanup before gas chromatography with electron capture detection (GC-ECD) analysis [21].

2. CONCLUSION

State of the art analytical techniques; diagnosis, quantitative amount, and therapeutic effects of antiviral drugs allow for a comprehensive understanding. In this way, more effective treatments will definitely emerge, despite the covid-19 epidemic that has been affecting our lives since 2019.

Conflict of Interest

Author has no personal financial or non-financial interests.

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Roadmap in Metabolomics Studies -A Mini Review

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ABSTRACT:
Omics strategies aim to identify all biomolecules (genes, proteins, metabolites, etc.) present in a biological tissue, cell, fluid or organism, generating large amounts of data that can be evaluated with the tools of biostatistics and bioinformatics. Metabolomics is an emerging field of science that has grown rapidly over the past few years. The metabolomics approach allows to detect differences in biological samples under normal conditions with disease, drug therapy, dietary intervention or altered states promoted by environmental modulation. In the beginning, metabolok studies were carried out on plants. however, with the developing technology and new analytical platforms, the focus has been on metabolites in the human body. As a result of developing methods, metabolomic imaging of biofluids, cells and tissues can be performed. With the development of metabolomic studies, it has become possible to diagnose diseases, understand disease mechanisms, identify new drug targets, customize drug treatments, and monitor therapeutic outcomes. In this review, results from three databases were used. 20 studies obtained from databases and reference lists were used.

Keywords : Analytical instruments, metabolomics, samples selection

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1.INTRODUCTION

Omics strategies are the field of study that does not aim to describe all of the biomolecules (genes, proteins, metabolites, etc.) present in a biological tissue, cell, fluid or organism, thus generating large amounts of data evaluated by biostatistics and bioinformatics tools.

metabolite; They are mostly polar biomolecules, which are formed in biological fluids as a result of metabolism reactions and have molecular weights less than 1000 Daltons, have different masses and chemical properties.

Metabolomics; It is the detection, quantification and identification of small molecule metabolites emerging from lipids, carbohydrates, vitamins, hormones and other cell components in tissues, cells and physiological fluids in a certain time period using high-throughput technologies.

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The first studies to include the term metabolomics were done in 1948 by Williams et al. carried out by [1]. In the study by Williams et al., metabolomics concepts were used with the data obtained by examining more than 200,000 paper chromatograms. In later studies, local terminological words were used to define different metabolism studies.

In 2001, Fiehn et al. used the term "metabolic fingerprint" to classify samples according to their origin and biological suitability [2]. Kell et al. (2005) proposed the term "Metabolomic footprint "to describe what a cell or system secretes under controlled conditions [3]. Link et al. (2015) used the term "real-time metabolome profile", which is used as "monitoring hundreds of metabolites with cycles of a few seconds in a few hours" in bacterial cells with high resolution mass spectrophotometer [4].

Before starting metabolomic studies, the study should be planned well. The firs parameter to be considered in the study is type of metabolic study. Then care should be taken to select the appropriate sample type for the study. The collection frequency of these collected samples and the number of times they will be collected and the storage conditions of samples should be determined before the study. If these collected samples are enzymatically active, it should be decided how to do this metabolic quenching. If the samples are to be stored for a long time, they should generally be kept −80 °C. the analytical platforms to be used after the sample collection phase should be selected. Each of these parameters must be defined before running [5, 6]. Apart from these parameters, a control sample and test samples are usually used to increase the accuracy of the results.

The data collected on the quality of metabolomic studies is of great importance. The selection, collection and preparation of sample samples has a high impact on the data obtained in order to make the studies better quality. For this reason, which sample will be collected in the study, how the samples will be extracted and how metabolic quenching should be done should be considered thoroughly. Studies are carried out in line with the analytical approach chosen according to the biological problem defined during the preparation of the sample.

Collected samples are divided into two groups. The first is metabolically inactive samples. these are usually the extracellular metabolome. does not require metabolic quenching and requires minimal sample preparation. the second is the metabolically active samples. They are usually found intracellularly and need metabolic suppression.

Quenching and extraction of metabolically active samples is one of the steps to be considered. Metabolic quenching of tissues is generally in the form of rapid washing in saline (saline solution) or phosphate buffered solution and rapid quenching by placing in liquid nitrogen [7].

Since it is specifically sought in targeted metabolomics studies, it must be optimized beforehand. For this, liquid-liquid or liquid-solid extraction can be done. Thanks to the extraction, the sample is cleaned. Sample preparation for

untargeted metabolomics studies is usually minimal. In chromatographic studies, sample dilution, filtration or protein precipitation is done. The high margin of error and time consuming in these steps affect the study negatively and limit the total number of samples.

2. SAMPLES

2.1. Urine

The collection and transportation of urine samples and the fact that they do not require trained personnel during collection are among the reasons why it is one of the most used biofluids. Since it is the excretory pathway of water-soluble metabolites and xenobiotics, the urinary metabolome is used to investigate the results of metabolomics for the whole body. In the study conducted by Bouatra et al, there are more than 3100 metabolites characterized in human urine [8]. The time to collect urine samples can make a significant qualitative and quantitative difference in the urinary metabolome [9, 10]. For this, the appropriate type of urine should be selected and collected.

2.2. Serum and plasma:

Serum and plasma are biological fluits that provide a snapshot of metabolism. It is a widely used biofluid in metabolomics studies. Because blood is in contact with many tissues in the human body and they contain a lot of information about human metabolism. The differences between serum and plasma have been investigated in studies, but not clear difference has been found between them. Only several minor differences were identified between the two samples [11-13]. Unlike the collection of most biofluids, personnel trained in blood collection must collect and process appropriate volumes of blood, and sample collection is therefore practiced in the clinic.

2.3. Saliva:

Although it is not used much, it is a suitable biological fluid for metabolic analysis. It is thought to accurately reflect the plasma metabolome. It is a preferred biofluid because it is easy to collect and noninvasive.

Apart from these, various biofluids such as cerebrospinal fluid, sweat, breast milk, primary and immortal cells are also used in metabolomic studies.

2.4. Tissues

Since human tissues are metabolically active, they need metabolic quenching quickly after gathering. Appropriately sized samples should be stored frozen at -80 °C. 20-100 mg of tissue sample is sufficient for a good reflection of the tissue metabolome [14, 15]. An important consideration for tissues is their inhomogeneity compared to biological fluids. For this, the tissues must be ground and extracted.

3. EXTRACTION

While the samples are being extracted, the aim is to draw the metabolites into the solvent system by selecting a suitable solvent. Liquit-liquit extraction is the most preffered extraction method. For this, different solvents such as methanol,

acetonitrile, isopropyl alchol, acetone and so on are used in extraction processes. Different ratios of these solvents are used depending on the type of operation. In addition to solvents, studies have sould that different tempatures and extraction times are important on extraction [16, 17].

4. ANALYTICAL INSTRUMENTS

4.1. Nuclear Magnetic Resonance

Although metabolomic experiments using NMR have been described since the early 1980s, the development of high-throughput NMR spectrometers with the necessary software for analysis is relatively new. The NMR method is a powerful tool for metabolomics studies offering reproducible analysis. With the newly developed spectrophotometers and software, it has been possible to perform high-efficiency analyzes with real-time feedback. Although there are many alternative NMR techniques, 1D-1H NMR is the most used technique.

4.2. Mass Spectrometry

The use of mass spectroscopy has been increasing in recent years. As a result of direct injection of the sample, ion suppression occurs due to the metabolite density in the environment. For this reason, a detection technique such as gas or liquid chromatography coupled to mass spectrophotometer is preferred. Mass analyzers can be used individually or in combination. Thus, fragment ions are fragmented again, allowing for more sensitive analysis. In this case, the experiment is called multistage mass spectrometry.

The most widely used method among all separation techniques is liquid chromatography coupled to mass spectrometry. The advantage of this method is its versatility. It allows the separation of various metabolites with various mobile and stationary phases [18]. The advantages of the method are its robustness, ease of use, ability to analyze various chemicals, and being suitable for targetless metabolomics analysis [19]. C_{18} and C_8 columns are preferred to obtain information about non-polar and weakly polar compounds. Hydrophilic interaction liquid chromatography (HILIC) is recommended for insufficiently charged hydrophilic, ionic and polar compounds [20].

Another method used is Gas Chromatography with Mass Spectrometry. An inert gas carries the evaporated sample to the heated capillary column. The capillary column is kept in an oven with fine temperature control. Metabolites with low boiling points are separated earlier. However, the disadvantage of this method is that it requires a derivatization step for some metabolites.

4.3. Combined Mass Spectrometry with Capillary Electrophoresis

It is one of the advantages of the method that it provides high efficiency and fast analysis without the need for preprocessing. Features such as the ability to evaluate polar or ionic compounds without requiring a derivation step have put CE in advantageous position. Each analytical platform used has its own advantages and disadvantages. In clinical applications, studies are usually done

using NMR and mass detector. As a result of studies with these platforms, complex data sets are obtained. This data requires various preprocessing before precessing.

5. CONCLUSION

With the discovery of metabolomics, most of the work initially focused on plants. As a result of the developing technology, clinical metabolomics, biofluids, molecular phenotyping of cells or tissues have begun to be considered. It is used in diagnosing diseases, understanding their mechanisms, identifying new drug molecules, customizing treatments and monitoring therapeutic results with metabolomics studies. It is thought that the studies carried out in these areas with the developing technology have high potential.

Conflict of Interest

Author has no personal financial or non-financial interests.

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