International Journal of PharmATA

2022; 2(1): 1-10

Research Article

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

Fatma Demirkaya Miloglu[1*], Tuba Saruhan[1]

[1] Department of Analytical Chemistry, Faculty of Pharmacy, Ataturk University, 25240

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

Received	Accepted	Published
01.12.2021	07.01.2022	11.01.2022

To cite this article:
Miloglu FD, Saruhan T. Square Wave and Differential Pulse Voltammetric Determination of Meloxicam in Pharmaceutical Formulations. International Journal of PharmATA. 2022; 2(1) 1-10

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].

* Corresponding Author
Tel : +90 442 231 5214
E-mail : fdkaya@atauni.edu.tr

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).

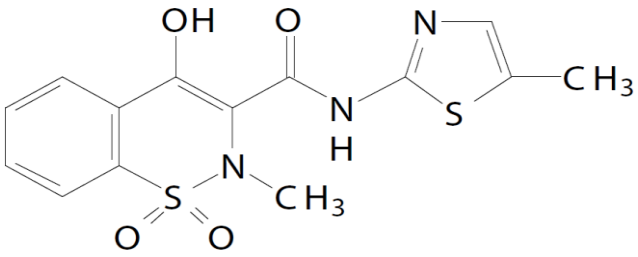

Figure 1. Chemical structure of MX

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**.

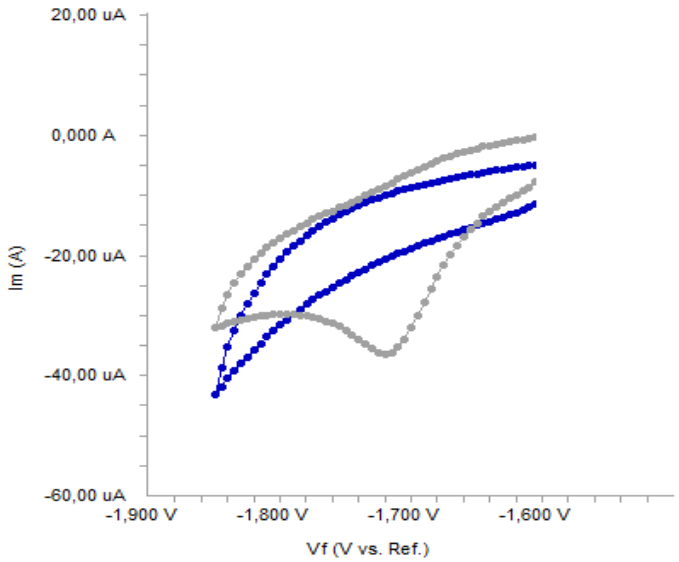

Figure 2. CVs of GCE in SEs (CH3COOH\CH3COONa buffer (0.2 M, pH 4.85)/NaOH (0.2 M) (v:v, 1:1) containing 50 µg/mL MX

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:

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

(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

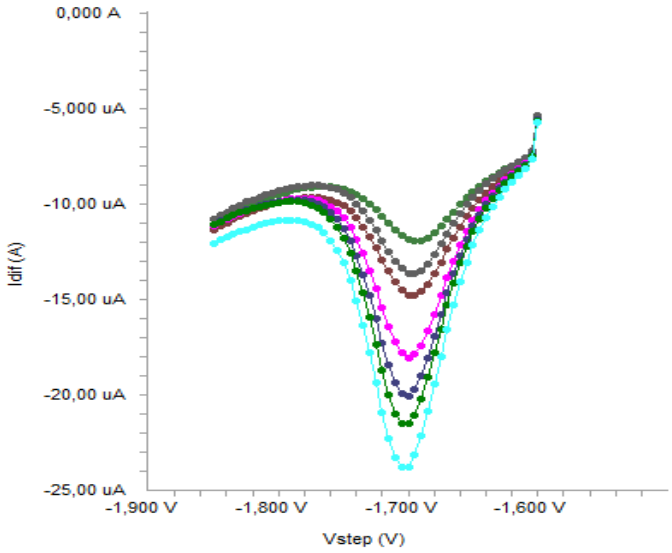

Figure 3. SWV voltammograms for different concentration of MX (10, 20, 40, 60, 70, 80 and 90 µg/mL) in SEs (CH3COOH\CH3COONa buffer (0.2 M, pH 4.85)/NaOH (0.2 M) (v:v, 1:1).

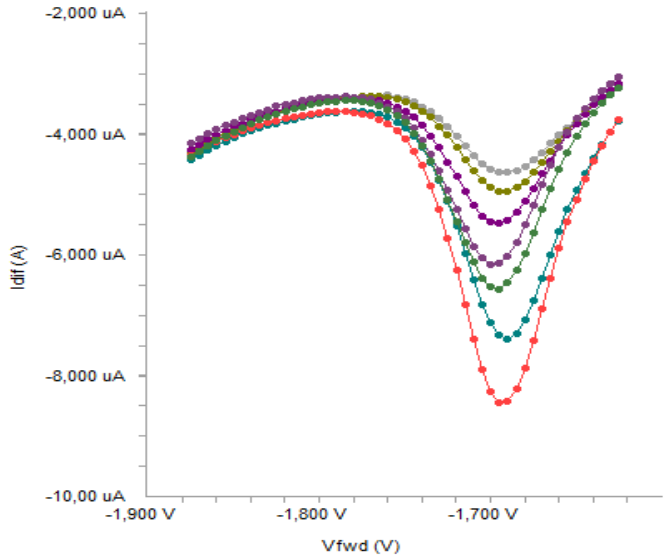

Figure 4. DPV voltammograms for different concentration of MX (10, 20, 40, 60, 70, 80 and 90 µg/mL) in SEs (CH3COOH\CH3COONa buffer (0.2 M, pH 4.85)/NaOH (0.2 M) (v:v, 1:1).

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.

REFERENCES

1. Phillips WJ, Currier BL, Analgesic pharmacology: II. Specific analgesics. JAAOS-Journal of the American Academy of Orthopaedic Surgeons. 2004; 12; 221-233.

2. Shekelle PG, Newberry SJ, FitzGerald JD, Motala A, O'Hanlon CE, Tariq A, et al, Management of gout: a systematic review in support of an American College of Physicians clinical practice guideline. Annals of internal medicine. 2017; 166; 37-51.

3. Soh J-W, Weinstein IB, Role of COX-independent targets of NSAIDs and related compounds in cancer prevention and treatment. Progress in experimental tumor research. 2003; 37; 261-283.

4. Pojarani LB, Zarifiazar S, Formulation and chracterization of meloxicam loaded niosome-based hydrogel formulations for topical applications. EMU Journal of Pharmaceutical Sciences. 2020; 3; 194-204.

5. Zawilla N, Mohammad MA-A, Aly SE-M, Determination of meloxicam in bulk and pharmaceutical formulations. Journal of pharmaceutical and biomedical analysis. 2003; 32; 1135-1144.
6. Garcia MS, Sánchez-Pedreño C, Albero MI, Marti J, Spectrophotometric methods for determining meloxicam in pharmaceuticals using batch and flow-injection procedures. European journal of pharmaceutical sciences. 2000; 9; 311-316.
7. Taha EA, Salama NN, Fattah LE-SA, Spectrofluorimetric and spectrophotometric stability-indicating methods for determination of some oxicams using 7-chloro-4-nitrobenz-2-oxa-1, 3-diazole (NBD-Cl). Chemical and pharmaceutical bulletin. 2006; 54; 653-658.
8. Bebawy LI, Stability-indicating method for the determination of meloxicam and tetracaine hydrochloride in the presence of their degradation products. Spectroscopy letters. 1998; 31; 797-820.
9. Hassan EM, Spectrophotometric and fluorimetric methods for the determination of meloxicam in dosage forms. Journal of pharmaceutical and biomedical analysis. 2002; 27; 771-777.
10. Taha EA, Salama NN, Abdel Fattah LS, Abdel Fattah, Stability-indicating methods for determination of meloxicam and tenoxicam in the presence of their degradation products. Spectroscopy letters. 2002; 35; 501-516.
11. Nemutlu E, Kir S, Method development and validation for the analysis of meloxicam in tablets by CZE. Journal of pharmaceutical and biomedical analysis. 2003; 31; 393-400.
12. Vignaduzzo SE, Castellano PM, Kaufman TS, Method development and validation for the simultaneous determination of meloxicam and pridinol mesylate using RP-HPLC and its application in drug formulations. Journal of pharmaceutical and biomedical analysis. 2008; 46; 219-225.
13. Madni A, Ahmad M, Usman M, Zubair MM, Shoiab H, Khan S, et al, New high performance liquid chromatographic method for simultaneous determination of diclofenac and meloxicam in oral formulation of liposomes and human plasma. Journal of the chemical society of Pakistan. 2010; 32; 654-661.
14. Altınöz S, Nemutlu E, Kır S, Polarographic behaviour of meloxicam and its determination in tablet preparations and spiked plasma. Il Farmaco. 2002; 57; 463-468.
15. Altiokka G, Atkosar Z, Tuncel M, Pulse polarographic determination of meloxicam. Pharmazie. 2001; 56; 184-185.
16. Radi A-E, Ghoneim M, Beltagi A, Cathodic adsorptive stripping square-wave voltammetry of the anti-inflammatory drug meloxicam. Chemical and pharmaceutical bulletin. 2001; 49; 1257-1260.
17. Beltagi A, Ghoneim M, Radi A, Electrochemical reduction of meloxicam at mercury electrode and its determination in tablets. Journal of pharmaceutical and biomedical analysis. 2002; 27; 795-801.

18. Radi A, El Ries M, El-Anwar F, El-Sherif Z, Electrochemical oxidation of meloxicam and its determination in tablet dosage form. Analytical letters. 2001; 34; 739-748.
19. Laviron E, Roullier L, Degrand C, A multilayer model for the study of space distributed redox modified electrodes: Part II. Theory and application of linear potential sweep voltammetry for a simple reaction. Journal of Electroanalytical Chemistry and Interfacial Electrochemistry. 1980; 112; 11-23.
20. Walfish S, Analytical methods: a statistical perspective on the ICH Q2A and Q2B guidelines for validation of analytical methods. BioPharm International. 2006; 19; 1-6.
21. El-Hefnawey G, El-Hallag I, Ghoneim E, Ghoneim M, Voltammetric behavior and quantification of the sedative-hypnotic drug chlordiazepoxide in bulk form, pharmaceutical formulation and human serum at a mercury electrode. Journal of pharmaceutical and biomedical analysis. 2004; 34; 75-86.