ANKARA UNIVERSITY FACULTY OF PHARMACY

ISOPS 13th International SYMPOSIUM ON PHARMACEUTICAL SCIENCES

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F2	Lu Turković	DEVELOPMENT OF A NOVEL HPLC-DAD-FLD-MS METHOD FOR THE SIMULTANEOUS DETERMİNATİON OF FIVE ANTICANCER DRUGS	Analytical Chemistry
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F25	Boğaç Buğra Barut	SIMULTANEOUS DETERMINATION OF SOME ANTIFUNGAL PESTİCİDES FROM HUMAN BIOLOGICAL SAMPLES BY HPLC	Analytical Chemistry

2D-ITP-CZE-MS/MS METHOD FOR ANALYSIS OF SEROTONIN IN URINE

[1]Matušková, M., [1]Čižmárová, I.,[1]Chaľová, P., [1,2]Mikuš, P., [3]Kováč, A., [3]Majerová, P., [4]Galba J.,[1,2]Piešťanský, J.

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Introduction:

Serotonin, chemically 5-hydroxytryptamine is monoamine with various biological functions. The major part of serotonin (95%) is synthesized from amino acid L-tryptophan in enterochromaffin cells in the gut. Serotonin interacts with receptors in the gastrointestinal tract, and affects gut functions (1). Serotonin can be found in platelets and also plays a role in inflammation and immune processes. Alterations in serotonin signaling have impact on inflammatory diseases – inflammatory bowel disease, allergic airway inflammation, or rheumatoid arthritis (2). In the CNS, serotonin acts as neurotransmitter. Its higher or lower concentrations in certain parts of brain are connected with neurologic and mental disorders (3).

Serotonin is therefore an interesting molecule and its concentrations in body liquids can provide valuable information. Typically, serotonin is determined in cerebrospinal fluid, plasma, and urine. Urine collection represents a noninvasive method without patient´s traumatization.

New 2D-CE method, coupling capillary zone electrophoresis (CZE) and isotachophoresis (ITP) with tandem mass spectrometry MS/MS detection was developed.

Materials and methods:

The capillary electrophoresis analyzer EA-102 (Villa Labeco, Spišská Nová Ves, Slovakia) was used in two-column coupling arrangement. The first column served for ITP analysis. It was provided with 800 µm inner diameter, 90 mm total length. It was made of polytetrafluoroethylene and connected with built-in contactless conductivity detector. The second one, 160 mm x 300 µm inner diameter polytetrafluoroethylene capillary, was used for CZE step.

Before each analysis, columns were manually rinsed with electrolyte. Samples were injected into the ITP capillary (via injection block) by the 10 µL Hamilton syringe. Hydrodynamically closed system is characterized by capillaries possesing larger inner diameter compared to hydrodynamically open system. Injected volumes can be therefore higher.

In the first – ITP capillary, sample was separated and continued into the second – CZE capillary. Between ITP and CZE was performed "a cut", which made main part of matrix without analyte not be allowed to enter the CZE capillary (see Fig. 1, upper trace)

ITP-CZE measurements were carried out in a constant current mode, at room temperature. The current in the first step was 300 µA, and 40 µA in the second step. Win ACES software, version 1.4 (Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia) was used to control of the analytical protocol.

The CE analyzer was equipped with triple quadrupole mass spectrometry detector (Agilent Technologies, Santa Clara, CA), with connection provided via the elution block (4).

Results and discussion:

he method was optimized and validated according to the US Food and Drug Administration guidance for bioanalytical method validation. In electromigration methods, analytes are separated in an electrolyte environment, therefore the choice of suitable electrolyte system plays cruical role in method optimization. Using mass spectrometry determines some characteristics of electrolyte systems: they should be volatile and have low ionic strength (5). Various combinations of electrolyte systems were tested (with NH_4Ac – ammonium acetate, NH_4Fo – ammonium formate, HAc – acetic acid, HFo – formic acid). Optimal composition of the electrolyte sestem was:

- **ITP stage** – LE (leading electrolyte) = 10mM NH_4Ac + 20mM HAc (pH = 4.5)
  TE (terminatig electrolyte) = 10mM HAc (pH = 3.3)
- **CZE stage** – BGE (background electrolyte) = 20mM HAc (pH = 3.1)

For method validation, working solutions of serotonin were prepared. Calibration solutions on eight concentration levels (0,08-41,43 ng/mL) were made by spiking serotonin standard into Surine™. The same matrix – Surine™ was used in preparation of quality control samples at three concentration levels (0,08286-20,714 ng/mL). Favorable parameters such as selectivity, linearity, precision, accuracy, and recovery, were achieved. Moreover, limit of detection (LOD) was found at very low, pg/mL concentration level. The developed method was successfully applied for the determination of serotonin in human urine samples.

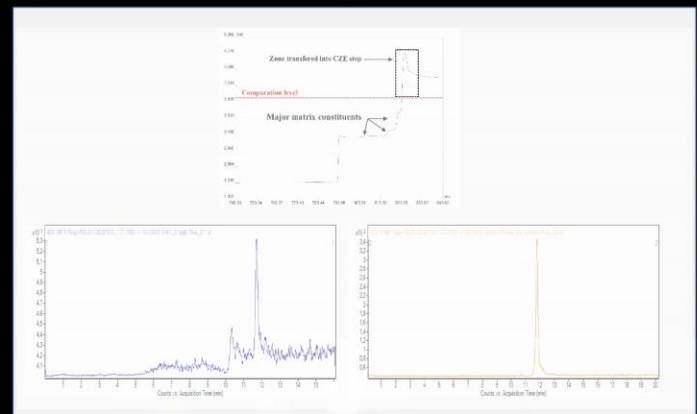

Fig.1. *Analysis of serotonin by ITP-CZE-MS/MS method.*
The upper figure *shows the first - ITP step, where the comparation level determines analytes, which enter CZE step (major matrix constituents are excluded). Sample is serotonin standard in Surine™ in low concentration level (near limit of quantification - LOQ).* ***The figure on the left*** *is MRM diagram illustrating CZE separation (with serotonin concentration near LOQ).* ***The figure on the right*** *is MRM diagram illustrating CZE separation of a human urine sample.*

Conclusions:

According to the obtained results, proposed method is an effective and sensitive tool for determination of serotonin in real biological samples. Combination of two electromigration methods, CZE and ITP, allows to determine serotonin in diluted human urine without time-consuming sample pretreatment. Method enables to detect very low concentrations of serotonin (at pg/ml level).

Acknowledgements

This study was supported by projects VEGA 1/0463/18, KEGA 027UK-4/2020, 388 APVV-15-0585, VEGA 2/0088/18, VEGA 2/0150/19, and APVV-18-0302.

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DEVELOPMENT OF A NOVEL HPLC-DAD-FLD-MS METHOD FOR THE SIMULTANEOUS DETERMINATION OF FIVE ANTICANCER DRUGS

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Introduction:
Palbociclib and ribociclib are novel anticancer agents used in advanced breast cancer treatment. Pharmacologically, they act as inhibitors of cyclin-dependent kinases 4 and 6 (CDKi) and are often combined with antiestrogen drugs such as anastrozole, letrozole or fulvestrant in order to enhance the overall survival (1). These therapeutic options are still relatively new and prone to inter-individual variabilities. Since the therapeutic outcomes in cancer treatment aren't immediately apprehensible while bearing a risk of toxicity at higher doses, it is possibly beneficial to monitor the plasma concentrations of these medicines (2). Therefore, sensitive and selective new bioanalytical methods, capable of determining extremely low concentrations of the analytes, need to be developed. In this work, chromatographic behaviour of the aforementioned five drugs was examined and appropriate detection methods established, in order to provide a basis for pharmacokinetic studies and therapeutic drug monitoring.

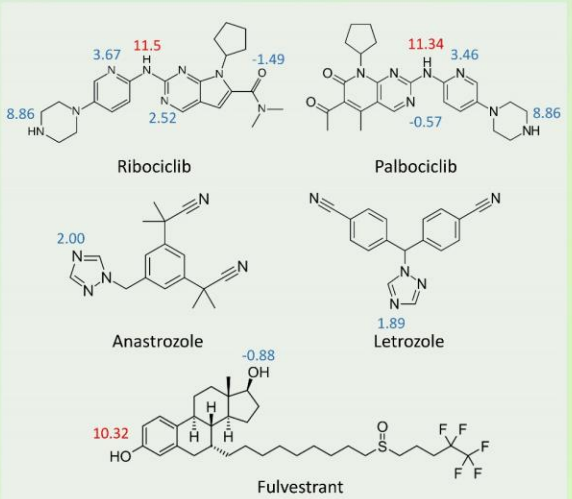
Materials and methods:
1 mg/mL standard stock solutions of ribociclib, anastrozole, letrozole and fulvestrant were prepared in methanol, while palbociclib was prepared as a 0.25 mg/mL solution in 50 % V/V acetonitrile. Sample solutions were prepared by mixing the appropriate volumes of the stock solutions and diluting to the final concentration with 65 % V/V methanol.
The samples were analysed using an Agilent 1100 high performance liquid chromatograph (HPLC) coupled with a diode-array detector (DAD) and a fluorescence detector (FLD) and a Waters Acquity ultra-high performance liquid chromatograph (UHPLC) coupled with a single quadrupole mass spectrometer (SQ-MS).
Five reverse-phase chromatographic columns were considered, as shown in Table 1. Gradient elution, with the tested mobile phase additives shown in Table 2, was applied. Methanol (MeOH) and acetonitrile (ACN) were examined as the organic mobile phase components. Flow rate was varied between 0.4 to 1.5 mL/min and temperature between 25 and 50 °C.

Table 1: tested chromatographic stationary phases

Column	Dimensions (mm)	Particle size (µm)
Merck LiChrospher C8	250x4	5
Agilent Zorbax C18	250x4.6	5
Waters XBridge C18	150x4.6	3.5
Waters XBridge Phenyl	150x4.6	3.5
Phenomenex Kinetex Biphenyl	150x4.6	2.6

Table 2: tested mobile phase pH values

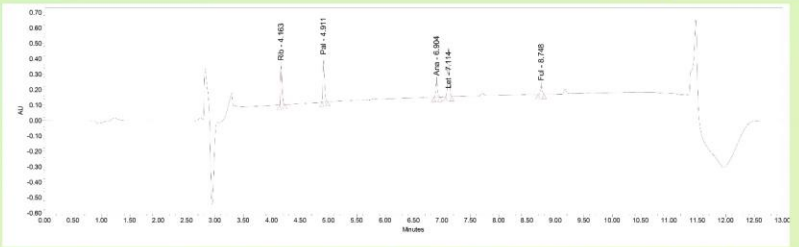
Additive in the aqueous phase	pH of the aqueous phase
0.1 % V/V phosphoric acid	2.3
0.1 % V/V formic acid	2.8
0.1 % V/V acetic acid	3.8
10 mM ammonium acetate buffer	5.3
10 mM ammonium bicarbonate	7.6



Picture 1: chemical structures of the analytes

Table 3: optimised gradient composition

t (min)	H_2O + 0.1 % HCOOH (% V/V)	ACN + 0.1 % HCOOH (% V/V)
0.0	85	15
8.5	0	100
10.0	0	100
12.0	85	15
20.0	85	15



Picture 2: representative chromatogram of a 50 µg/mL standard solution of all five analytes in 65 % V/V methanol under optimised conditions, detected by DAD at 220 nm

Table 4: notable chromatographic parameters obtained under optimised conditions

Name	Retention Time (min)	Resolution	DAD absorption maximum (nm)	DAD limit of detection (µg/mL)	FLD limit of detection (µg/mL)	Main ion ESI+ (m/z)	MS limit of detection (ng/mL)
Ribociclib	4.163		270	0.03	-	435.45	52.2
Palbociclib	4.911	15.0	366	0.11	-	448.41	40.0
Anastrozole	6.904	37.9	210	1.24	0.91	294.27	19.8
Letrozole	7.114	3.8	240	0.10	0.03	217.17	16.6
Fulvestrant	8.748	28.2	210	1.00	0.34	607.67	2.0

Results and discussion:
Chemical structures of all analytes, along with their pKa values predicted by ChemAxon, are shown in Picture 1.
Due to favourable column chemistries, acceptable results were obtained using phenyl and biphenyl stationary phases. The biphenyl column showed best resolutions and peak shapes in the shortest analysis time so it was therefore selected as optimal.
Alkalisation of the mobile phase showed deleterious effects on the peak shapes and resolutions of the weakly basic CDKi. As expected, acidic conditions proved optimal, as the adsorption of the positively charged CDKi to any remaining deprotonated silanol groups was minimalised, while they also eluted early owing to their high degree of ionisation. 0.1 % *V/V* formic acid, added to both the aqueous and the organic phase, was selected due to its MS-compatibility.
Methanol and acetonitrile were tested as the organic mobile phase components. Even though methanol is recommended over acetonitrile with phenyl-based stationary phases, acetonitrile showed superior results and was used, with the final gradient conditions depicted in Table 3.
Flow rate of 0.5 mL/min was chosen to enable splitless MS analysis, and the column was thermostated at 25 °C, since higher temperatures didn't prove beneficial.
DAD was used in preliminary studies, with the analytes' maximum absorption wavelengths presented in Table 4. It was also established that anastrozole, letrozole and fulvestrant could be determined using FLD. Since anastrozole showed the weakest fluorescence, but still higher LOD-s than on DAD, the detection wavelengths (excitation at 210, emission at 310 nm) were set accordingly. Fulvestrant and letrozole could be simultaneously determined at the same wavelength pair. The detection limits achieved on DAD and FLD are also shown in Table 4.
An SQ-MS was finally used to evaluate the most prominent ions, listed in Table 4 along with the achieved detection limits.

Conclusion:
A new HPLC-DAD-FLD-MS method for the simultaneous determination of palbociclib, ribociclib, anastrozole, letrozole, and fulvestrant, was developed. The optimal conditions include: a 150x4.6 mm, 2.6 µm biphenyl column thermostated at 25 °C, mobile phase in gradient elution at 0.5 mL/min flow rate, consisting of water and acetonitrile, both containing 0.1 % V/V formic acid. The representative chromatogram is shown in Picture 2, with the chromatographic parameters listed in Table 4. The proposed method could further be utilised for plasma sample analysis. Appropriately low limits of detection and quantification should be achieved using MS detection, and, if necessary, sample preconcentration.

Acknowledgments:
This work has been fully supported by the Croatian Science Foundation under the project number UIP-2019-04-8461.

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STUDY OF SPONTANEOUS REGRESSION OF CANCER AND SUBSEQUENT USE OF ADVANCED ANALYTICAL METHODS

[1,2]Chaľová, P., [1]Matušková, M., [1]Čižmárová, I., [1]Mikuš, P., [2]Minichová, L., [2]Škultéty, L., [2]Lakota, J., [1]Piešťanský, J., [2]Galba, J.
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Introduction

Spontaneous regression of cancer is defined as the partial or complete disappearance of a malignant tumor without any treatment or therapy which is considered significant impact on neoplastic disease (1). The mechanism of this phenomenon is still unknown and therefore it is a great challenge for our research team to use advanced analytical methods to help understand its nature. We assume that research of metabolome and proteome will significantly clarify this phenomenon.

Materials and Methods

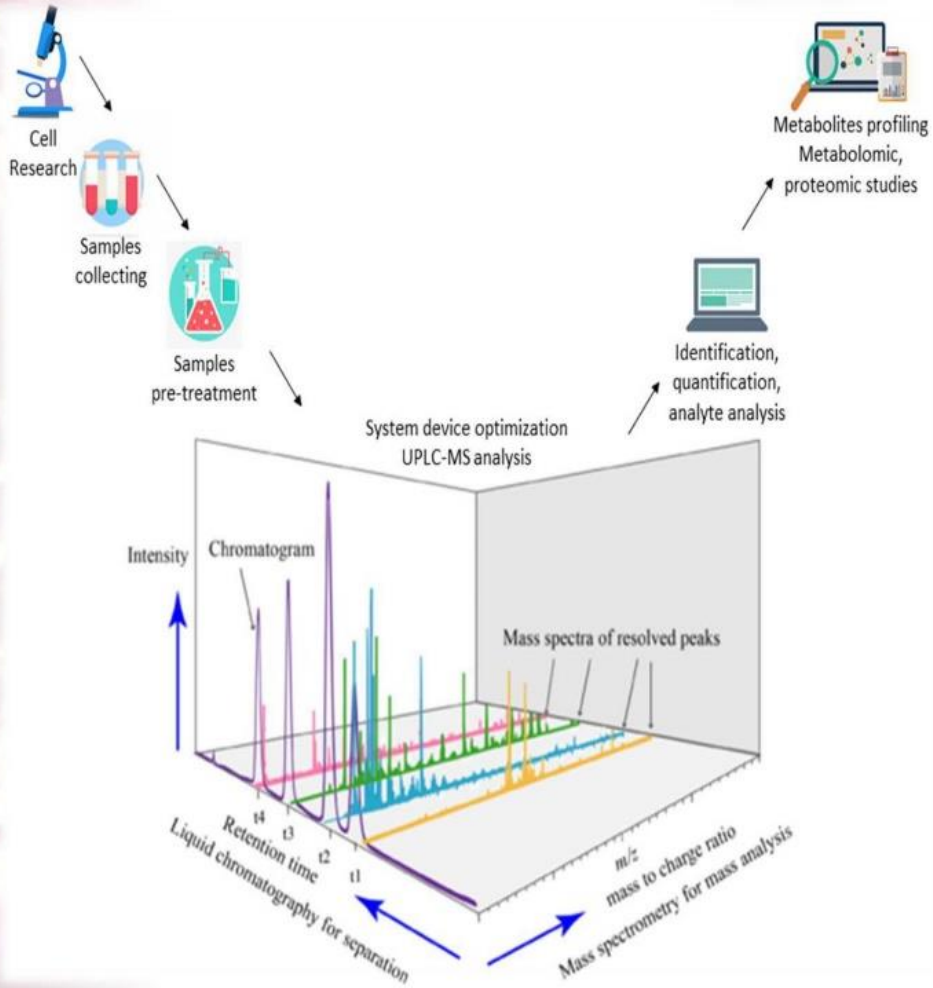
In the cell research we used PC3 cells, cultivated on DMEM medium, trypsin passaged and treated with: a) sera from patients with spontaneous tumor regression, b) mouse sera with anti-CAI antibody, c) pure anti-CAI antibody isolated from human erythrocytes. In the analytical research after collecting samples from cell research, we will use UHPLC in combination with detection techniques based on HRMS, MS/MS.

Discussion

An interesting fact in this area is the presence of antibodies against carbonic anhydrase I in the sera of some patients. It should be noted that the presence of these antibodies was correlated with an increased probability of survival (2). Our research team also proved this fact. In the future we will focus on metabolomics research, and we will try to understand the mechanism of spontaneous tumor regression, to discover new oncomarkers and develop prognostic or therapeutic tools for oncological diseases.

Results

In the cell research, we observe that the simulation of spontaneous tumor regression with three types of treatment has a positive significant effect on PC3 cells-presence of cytopathic effect.



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This study was supported by a grant of Comenius University UK/235/2021, grant VEGA 1/0463/18, grant APVV-18-0340.

DEVELOPMENT OF CE-MS METHOD FOR ANALYSIS OF TRIPTORELIN

[1]Čižmárová, I., [1]Matušková M., [1]Chaľová P., [1,2]Mikuš, P., [3]Galba J., [1,2]Piešťanský J.

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INTRODUCTION

Triptorelin is a synthetic decapeptide (pGlu-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-Pro-Gly-NH2) which, depending on the treatment protocol, may act as an agonist or antagonist at gonadotropin-releasing hormone receptors (GnRH). It causes secretion of the luteinizing hormone (LH) and the follicle-stimulating hormone (FSH) from the pituitary gland. LH and FSH induce the synthesis of testosterone and estrogen. The pharmacological antagonism of triptorelin at GnRII receptors is mainly used in the treatment of hormone-responsive breast cancer, as a palliative treatment for prostate cancer, in the treatment of endometriosis, sexual deviations in men, and the premature onset of puberty. Lower doses and longer dosing intervals enhance its agonistic effect at the receptors what is abused by athletes to achieve better sports performance or to prevent side effects caused by long-term anabolic use. The gonadotropin-releasing hormone and its analogs (buserelin, gonadorelin, triptorelin, etc.) increase testosterone levels in the body, and therefore they are referred as growth promoters and were included in the list of banned substances in 2014 (1).

INSTRUMENTATION

The CE experiments were carried out on a CE Agilent 7100 system coupled online with Triple Quadrupole tandem mass spectrometer. Fused silica capillary (ID 50 µm) with the lenght 700 mm was used for all measurements. Each day before the start of the measurement the electrolyte was changed, the capillary was flushed with water for 10 minutes and then with the background electrolyte for 10 minutes as well. The separation was performed in a cationic separation regime, the applied voltage was set at +25 kV and the driving current was 60 µA. Two characteristic m/z transitions were applied in the MRM mode for unequivocal identification and quantification of triptorelin: 656.5 → 328.3 (quantification transition), and 656.5 → 249.0 (identity confirmation transition) (Fig. 1).

STANDARD & SAMPLES SOLUTIONS

Chemicals were purchased in analytical quality from Merck (Darmstadt, Germany), Sigma Aldrich (Steinheim, Germany), and Fluka (Buchs, Switzerland). Triptorelin was purchased from Caslo (Lyngby, Denmark). The stock solution of triptorelin was prepared by dissolving 0.1 mg of triptorelin in 1 mL of demineralized water. Working solutions of triptorelin were in the range of 0.05-10 µg/mL (0.05; 0.1; 0.5; 1, 2, 5; 10) were prepared by proper dilution of the stock solution by demineralized water.

Table 1: Separation parameters for triptorelin in the optimization process in the hydrodynamic injection mode.

BGE	t_m [min]	Area	Height	FWHM	S/N
1M HFo	28.863	5440.67	209.39	0.404	328.7
10mM HFo	25.384	9213.21	285.71	0.481	378.3
20mM HFo	25.724	9517.2	334.25	0.413	499.5
50mM HFo	26.674	10958.6	335.6	0.437	397.8
10mM NH_4Fo + 20mM HFo	22.585	9282.79	320.25	0.375	412.8
20 mM HFo + 10% MeOH	21.702	2000	78.67	0.174	131.0

Figure 1: Product ion MS spectrum of triptorelin.

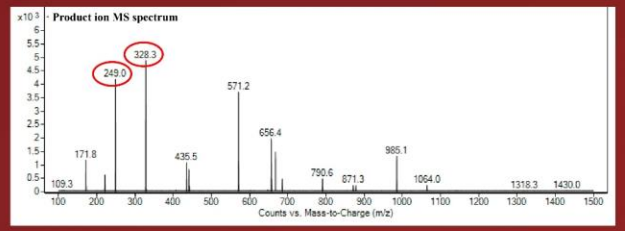


Figure 3: Comparison of hydrodynamic (green) and electrokinetic (orange) injection. Experiments performed with 70 cm long separation capillary. Concentration of triptorelin was 50 ng/mL.

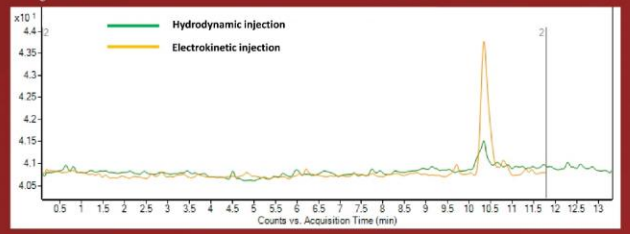
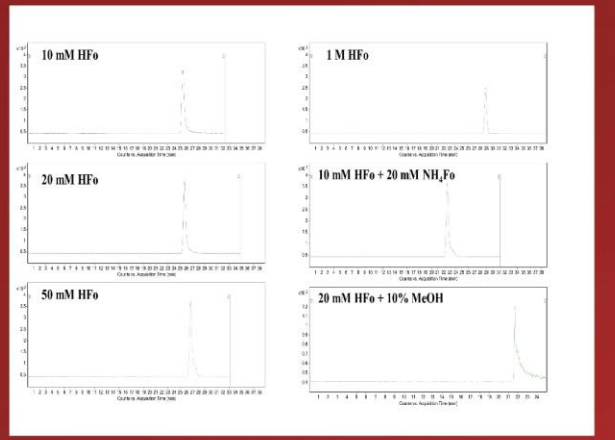


Figure 2: Effect of BGE composition on triptorelin separation. Experiments performed with 120 cm long separation capillary



RESULTS

In the optimalization process the composition of the background electrolyte (BGE) was investigated. Various MS compatible chemical were used, such as formic acid (HFo), acetic acid (HAc) and their ammonia salts (Table 1). From the tested BGEs, the best separation conditions (migration time, signal intensity, S/N ratio, separation efficiency) were obtained with the use of 20mM HFo (pH= 2,69) (Table 1, Figure 2). We also tested the addition of alcohols to the background electrolyte. These additions of alcohols resulted in shorter migration time but also lower analytical signal intensities and peak tailing (Fig. 2). To improve the sensitivity of the method we tested two different types of injection – hydrodynamic and electrokinetic. Electrokinetic injection of the sample (FASS), known as online pre-concentrating method, was characterized by enhanced intensity of the analytical signal. Such approach led to more then 10 times higher analytical signal when compared to measurements with convenient hydrodynamic injection (Fig. 3). The predicted limit of detection (LOD) values were 50 ng/ml (for hydrodynamic injection) and 10 ng/mL (for electrokinetic injection).

CONCLUSION

In this work, a fast, sensitive, cheap, and ecological analytical CE-MS method for the analysis of triptorelin was developed. Crucial separation and detection parameters were optimized. Favorable separation and operation parameters were obtained. The predicted LOD and LOQ values are 50 times lower than up to the present published values. The presented method represents an effective tool for monitoring triptorelin in model samples.

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ACKNOWLEDGEMENTS

This study was supported by the projects VEGA 1/0463/18 and KEGA 027UK-4/2020

ELECTROANALYTICAL ANALYSIS OF GUAIFENESIN ON POLY(ACRIDINE ORANGE) MODIFIED GLASSY CARBON ELECTRODE AND ITS DETERMINATION IN PHARMACEUTICALS AND SERUM SAMPLES

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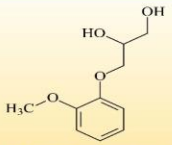
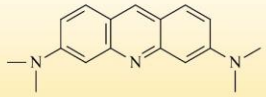


Guaifenesin [(R,S)-3-(2-methoxyphenoxy)-propane-1,2-diol], is an expectorant that is widely used to treat cough and congestion caused the common bronchitis, cold and other breathing illnesses. Guaifenesin provides thinner mucus, increments the lubrication of the respiratory tract (lungs, nose, and throat) and increases the removal of mucus. Also, it used in surgery owing to its additive effect on narcotics and its activity as a muscle relaxant [1].



Acridine orange (AO), is an aromatic dye containing nitrogen which is generally used in cell biology [2]. Due to the structure and molecular recognition capability of AO, it is can be easily polymerized electrochemically on the surface of solid electrodes as a redox mediator for the determination of electroactive molecules.

Method: Differential pulse adsorptive stripping voltammetric method for determination of guaifenesin on a poly(acridine orange) modified glassy carbon electrode has been developed. Glassy carbon electrode was modified with electropolymerization of acridine orange monomer for sensitive determination of guaifenesin. Guaifenesin provided highly reproducible and well-defined irreversible oxidation peaks at +1.125 V and +1.128 V (*vs.* Ag/AgCl) in the selected supporting electrolyte and human serum samples, respectively.

Polymerization of AO: AO was polymerized electrochemically on the surface GCE by potential cycling for 20 cycles in the potential range between -0.3 and +1.2 V versus Ag/AgCl in 25 mM PBS at pH 5.5 [3].

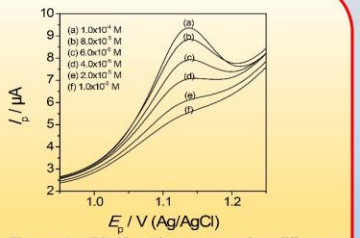


Figure 1. DPAdS voltammograms for different concentrations of GUF in 0.04 M BRB solution at pH 7.0 on the PAO modified GC electrode

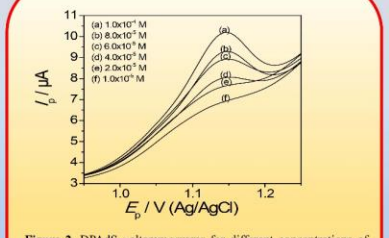


Figure 2. DPAdS voltammograms for different concentrations of GUF in spike serum samples on the PAO modified GC electrode

Table 1. Validation data of calibration lines for the quantitative determination of GUF by DPAdSV on the PAO modified GC electrode.

	Supporting electrolyte	Spiked serum
	DPAdSV	DPAdSV
Peak potential (V)	1.125	1.128
Lincarity range (µM)	0.200-100	0.400-100
Slope (µA µM^{-1})	$3.5\times10^{-2}\pm1.11\times10^{-3}$	$3.28\times10^{-2}\pm7.027\times10^{-4}$
Intercept (µA)	$-0.286\pm1.25\times10^{-2}$	$0.588\pm2.799\times10^{-2}$
Correlation coefficient	0.999	0.995
Limit of detection (µM)	5.779×10^{-3}	4.794×10^{-2}
Limit of quantification (µM)	0.018	0.145
Repeatability of peak current (RSD%)	0.422	0.418
Repeatability of peak potential (RSD%)	0.203	0.197
Reproducibility of peak current (RSD%)	0.657	0.848
Reproducibility of peak potential (RSD%)	0.248	0.242

Table 2. The results of the determination of GUF from its pharmaceutical dosage form and recovery experiments in 0.04 M BRB solution at pH 7.0 by DPAdSV on the PAO modified GC electrode

	Syrup (200 mg / 15 mL)
	DPAdSV
Labelled claim (mg)	200.00
Amount found (mg)*	199.71
RSD%	0.48
Bias%	-0.145
Added (mg)	100
Found (mg)*	100.367
Average recovered (%)	100.367
RSD% of recovery	0.667
Bias%	0.367

*Each value of the mean 5 experiments.

Table 3. The results for the determination of GUF from spiked human serum samples.

	DPAdSV
Added concentration (M)	6.00×10^{-5}
Obtained concentration (M)*	6.022×10^{-5}
Average recovered (%)	100.367
RSD% of recovery	0.667
Bias%	0.366

*Each value of the mean 5 experiments.

Table 4. Compared parameters obtained using different electrochemical sensors for the determination of GUF.

Electrode	Method	Linear range (M)	LOD (M)	Ref.
PCF	DPV	1.0×10^{-7}- 2.5×10^{-5}	2.3×10^{-8}	[4]
MWCNT/IL/GCE	DPV	1.5×10^{-6}- 4.8×10^{-4}	8.5×10^{-5}	[5]
Au-PtNPs/NFs/CNT/GCE	DPV	5.0×10^{-8}- 3.0×10^{-4}	1.75×10^{-8}	[6]
Platin electrode	DPV	1.0×10^{-4}- 3.03×10^{-4}	-	[7]
PAO modified GC electrode	DPAdSV	2.0×10^{-7}- 1.0×10^{-4}	5.779×10^{-9}	This work

PCF: Anodized nanocrystalline graphite-like pyrolytic carbon film electrode
MWCNT/IL/GCE: Multiwalled carbon nanotube-ionic liquid modified glassy carbon electrode
Au-PtNPs/NFs/CNT/GCE: Carbon nanotube bimetallic Au-Pt inorganic-organic nanofiber hybrid nanocomposite electrode

CONCLUSION

In this study, CV and DPAdSV methods were used for voltammetric analysis of GUF in the pharmaceutical dosage forms and human serum samples. The linear response was obtained from 2.00×10^{-7} to 1.00×10^{-4} M with a detection limit of 5.779×10^{-9} and a correlation coefficient of 0.999 by DPAdSV. The detection limit was obtained lower than the voltammetric methods reported in the literature. Also, the repetitive replies for the peak current and the peak potential of GUF were obtained with DPAdSV on the PAO modified GC electrode. As a result, the presented method offered high sensitivity and selectivity for the analysis of GUF in pharmaceutical formulations and biological samples without the requirements of sample pre-treatment or time-consuming extraction and evaporation steps before to the analysis.

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THE NOVEL APPROACH TOWARDS GRADIENT ELUTION HPLC METHOD DEVELOPMENT

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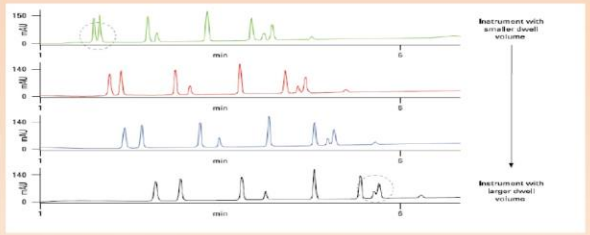
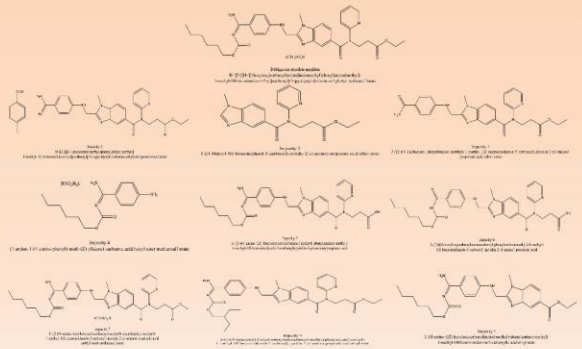
INTRODUCTION

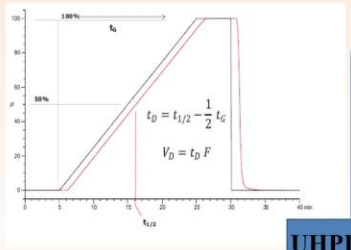
Gradient elution HPLC finds its purpose in simultaneous analyses of solutes covering wide range of polarities. However, the instrument related factors, especially dwell volume, are frequently responsible for fizzy transfer and short life cycle of the gradient elution method. Therefore, it is advisable to incorporate dwell volume into the optimization stage and avoid transfer related failures. The chemometric approach would enable selection of optimal chromatographic conditions for different HPLC instruments. The aim of this study was to propose and test this approach in gradient elution method's development. Dabigatran etexilate mesylate and nine structurally related compounds were selected as suitable model mixture due to its complexity and polarity.



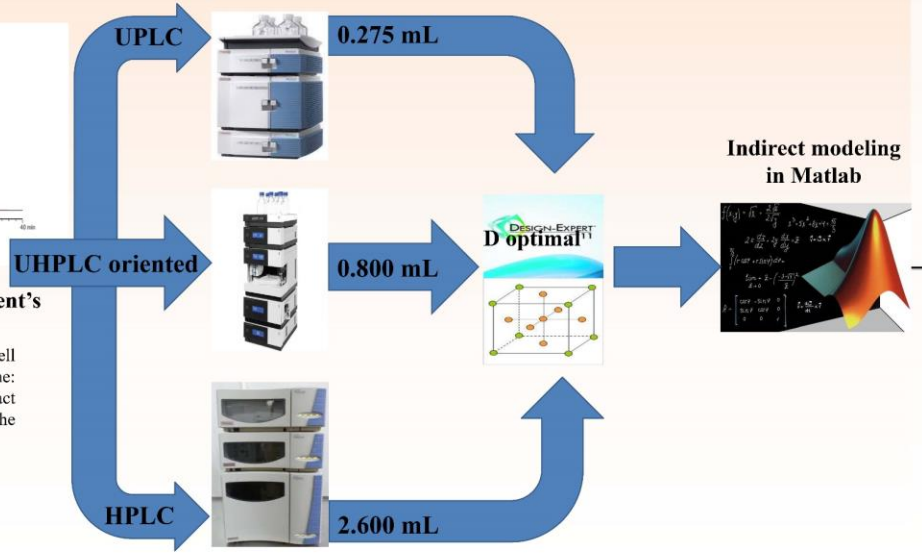
Example of dwell volume impact on chromatographic separation
(Eliminate your application and chromatography challenges, LC Scientist session ,Agilent June 2020)



MATERIAL S AND METHODS

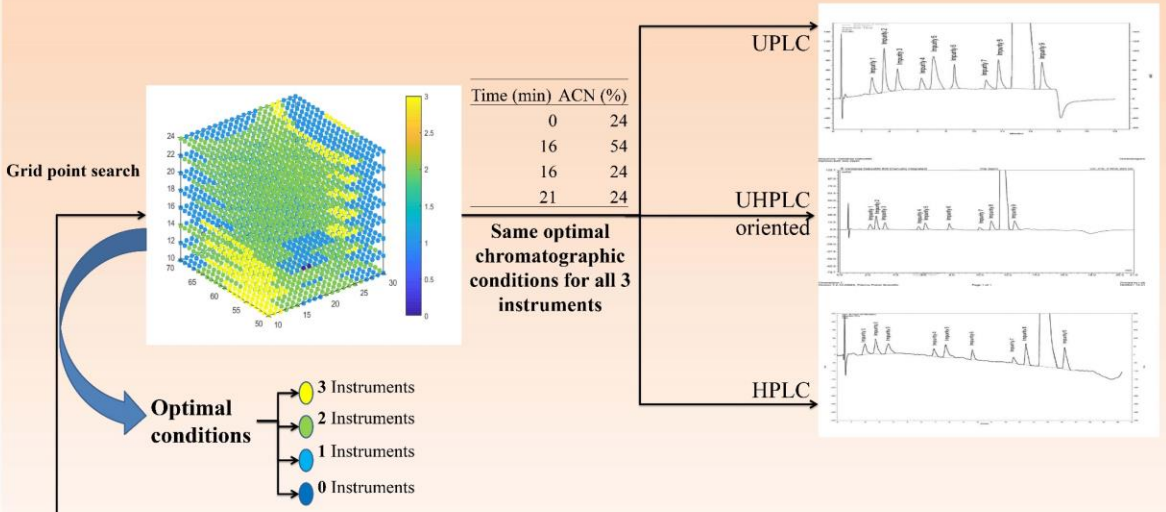


Determination of instrument's dwell volume
(P. Hong, P. R. McConville, Dwell volume and extra column volume: What are they and how do they impact method transfer?, Waters – The Science of what's possible, 2018.)



RESULTS

Method development was supported with an experimental design methodology, namely Placket – Burman for screening and D-optimal design for optimization purposes. Dwell volumes were included in the optimization phase and in this way the same optimal chromatographic conditions for all three instruments were selected. They included 10 mM ammonium acetate buffer with pH set to 4.9 using acetic acid, and acetonitrile. The components of the mobile phase were pumped into chromatographic system with flow rate of 400 µL min^{-1} in a linear gradient mode: at 0 minutes 24% (v/v) acetonitrile and 76% (v/v) of buffer solution, at 15 minutes 54% (v/v) acetonitrile and 46% (v/v) buffer solution. At 16 minutes the acetonitrile content was back to 24% (v/v) and 76% (v/v) of buffer solution. The re-equilibration time was set to 5 minutes. The examined chromatographic region is graphically presented and optimal conditions are noticed as the cross sections (yellow dots).The method was validated and confirmed its utility on all instruments.



CONCLUSION

The proposed methodology demonstrated its ability to predict joint optimal chromatographic conditions for instruments with different values of dwell volume. The potential was confirmed on complex model mixture and instruments significantly differing in dwell volume values. In this way the gap between developing and routine needs could be overwhelmed, followed by facilitated transfer of methods.

ACNOWLEDGEMENTS / CONTACTS

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Project no. 451-03-9/2021-14/200161).

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CHEMOMETRICALLY SUPPORTED OPTIMIZATION OF RP/WCX-HPLC METHOD

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INTRODUCTION

- active pharmaceutical ingredients are often in **salt form**
- weak cation exchange (**WCX**) interactions in addition to reverse-phased (**RP**) hydrophobic could improve separation
- method development was governed using Design of Experiments (**DoE**) – evaluation of multifactorial influance simultaneously
- **Derringer's desirability function** - used for multiobjective decision making during method optimization

AIM

- to elucidate experimental factor influence in **mixed mode WCX/RP** bimodal system on retention of diverse ionized APIs
- to efficiently optimize mixed mode LC method

MATHERIAL AND METHOD

Analytes:

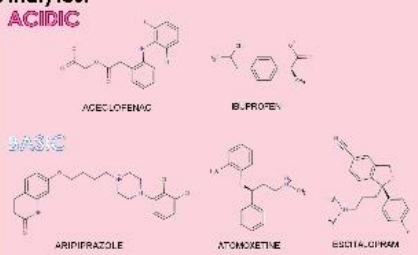

Figure 1. Graphical presentation of analytes' structures

Equipment:

- Dionex 3000, LC
- Thermo Acclaim Mixed Mode WCX-1 column (3 µm; 2,1 x 150 mm)

Figure 2. Schematic presentation of stationary phase and analytes structure

Design of experiments:

Table 1. Experimental factors for mobile phase composition

EXPERIMENTAL PARAMETERS	LEVELS		
	-1	0	+1
ACN (v/v %)	30	40	50
pH (acetic buffer)	3.8	4.7	5.6
I (acetic buffer)	20	30	40
Temperature (C)	30	34	38

- Screening phase: **Full Factorial Design 2^4** (levels: -1, +1)
- Optimization phase: **Central Composite Design – face-centered** (levels: -1, 0, +1)
- Design-Expert 7.0.0 used for obtaining experimental plan and mathematical models

RESULT AND DISCUSSION

SCREENING PHASE

Acetonitrile (ACN) content:

- the greatest impact on analytes' retention factors (k)
- increase in ACN content caused a decrease in k

Temperature (T):

- same as ACN content influence
- much less pronounced

pH:

- opposite effect on anionic and cationic species
- greater ionization of stationary phases' carboxylic groups at higher pH
- repulsive interactions with anionic species
- attractive interactions with the cationic analytes are enhanced, vice versa

Ionic strength (I):

- stronger influence on cationic analytes than on anionic ones

OPTIMIZATION PHASE

- All of four factors were included during optimization phase
- Optimization goals:
  - k (IBUPROREN) in range 1–10
  - k(ARIPIPRAZOLE)<10
  - k(ACECLOFENAC)>1
  - α(ATOMOXETINE/ESCITALOPRAM)>1.3.
- Statistical evaluation of mathematical models:
  - R2, adj. R2, pred. R2 > 0.95
- Derringer's desirability function – set of optimal conditions:
  - **47% (v/v) ACN**
  - **ACETIC BUFFER (40 mM, pH 3.8)**
  - **TEMPERATURE 30 °C**

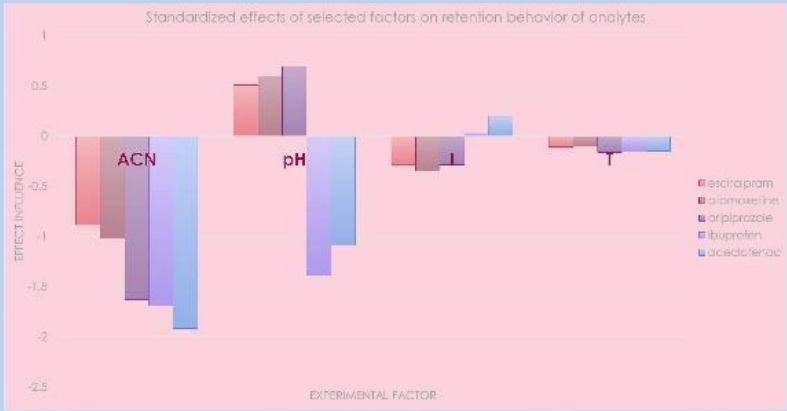

Figure 3. Graphical presentation of experimental factor's standardized effects on behavior of examined analytes in mixed mode RP/WCX system

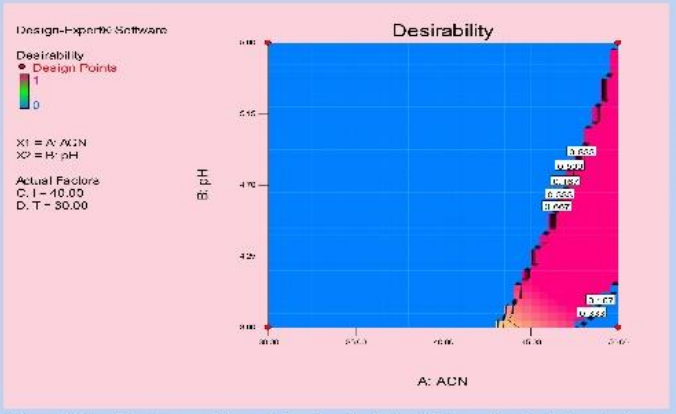

Figure 4. Graphical presentation of Derringer's desirability function for determination of condition values for optimal separation of analytes

CONCLUSION

- experimental parameters with significant influence on retention in bimodal RP/WCX system were evaluated and included **ACN (v/), pH, I, T**
- method was successfully optimized
- optimal factor values were selected to be **47% (v/v) ACN,40 mM acetic buffer with pH 3,8 and column temperature of 30 °C**

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Acknowledgements
This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (451-03-9/2021-14/200161).

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DETERMINATION OF ORNIDAZOLE IN PHARMACEUTICAL DOSAGE FORMS USING BSA COATED FLUORESCENT COPPER NANOCLUSTER

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INTRODUCTION

Ornidazol, ORN, (1-(3-chloro-2-hydroxy)propyl-2-methyl-5-nitroimidazole or 1-chloro-3-(-2-methyl-5-nitroimidazole-1-yl)propan-2-ol) is member of third-generation nitroimidazoles which has antiprotozoal and antibacterial properties.
Metal nanoclusters, which are luminescent materials, are very promising compared to organic molecules. Metal nanoclusters consisting of several to hundred atoms, they exhibit molecular-like properties such as the HOMO-LUMO transition, stereochemical activity, redox property, and intrinsic magnetism. In this study, a new method has been developed by using BSA coated copper nanoclusters (CuNCs) for the determination of ORN from pharmaceutical dosage forms. Characterization of CuNCs and experimental parameters were made with the following techniques: fluorescence, UV-Vis, FT-IR, TEM, zeta potential measurement and dynamic light scattering.

EXPERIMENTAL

Instruments

Fluorescence measurements were done by using Varian Cary Eclipse spectroflorimeter equipped with a Xenon lamp and 10 × 10 mm path length quartz cells. Excitation and emission slits were 10.0 nm and excitation wavelength was set at 325 nm. UV-Vis measurements were recorded with Specord 50 Plus (Analytik Jena, Germany). FEI Tecnai G2 Spirit Biotwin CTEM was used to obtain TEM images. Perkin Elmer Spectrum 400 FTIR / FTNIR spectrometer equipped with a Universal ATR Sampling Accessory (Perkin Elmer Inc., Waltham, Ma, USA) was used for FT-IR studies and were reported in cm^{-1} units. Zeta potential and Dynamic Light Scattering (DLS) analysis were carried out on a Zetasizer Nano ZS Series, Malvern instrument. X-ray Photoelectron Spectroscopy (XPS) analysis were done by using PHI 5000 VersaProbe III multitechnique XPS (ULVAC-PHI, Japan). pH measurements were made with a combined pH electrode with a Mettler-Toledo GmbH (Greifensee-Switzerland) pH meter. All experiments were carried out at room temperature.

Synthesis of BSA lemplated CuNCs

BSA coated CuNCs were synthesized by minor modification according to the method of Goswami et al. [19]. Briefly, aqueous $Cu(NO_3)_2.3H_2O$ (1 ml, 20 mM) was added to BSA solution (5 mL, 15 mg/mL). The solution was stirred at room temperature for 5 minutes. Then, pH was adjusted to 12 by adding NaOH. Upon reaching pH 12, the color of the solution changed from pale green to purple. Finally, the mixture was stirred vigorously at 55 ° C for 7 hours. After the time the color of solution changes to light brown. Synthesized nanoclusters were stored in the refrigerator.

Interaction between ORN and Copper Nanoclusters

Before interaction studies, the effect of pH on ORN and CuNCs was examined. Phosphate buffer solutions at different pH values were tested.

In order to evaluate of interaction between ORN and CuNCs, 2.0 mL solution 0.1 M pH 12 phosphate buffer in 1.0 cm quartz cell, containing appropriate concentration of CuNCs, was titrated by successive additions of a stock solution of ORN. Titration was done manually by using a micropipette. The fluorescence emission spectra were then measured in the wavelength range of 335-500 nm with exciting wavelength at 325 nm in the presence and absence of ORN. The quenching of fluorescence intensity was recorded as a ratio F_0 / F, where F_0 and F represent the fluorescence intensities of the probe in the absence or presence of ORN. All measurements were carried out three times and obtained average values were used for the calculations.

RESULTS

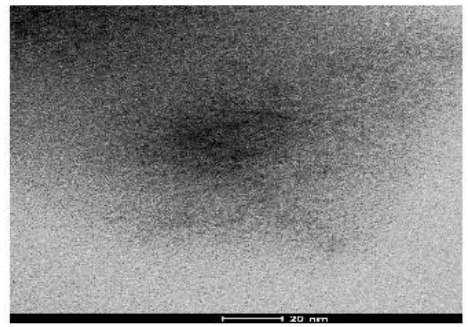

Figure 1. TEM image of CuNCs

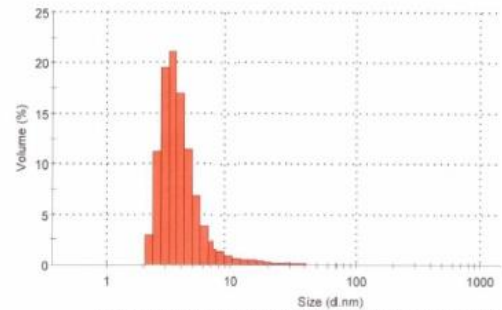

Figure 2. Dynamic light scattering result of CuNCs

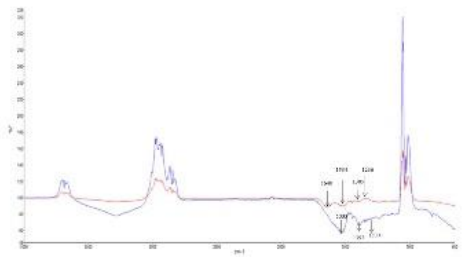

Figure 3. FT-IR spectrum of BSA (red line) and CuNCs (blue line)

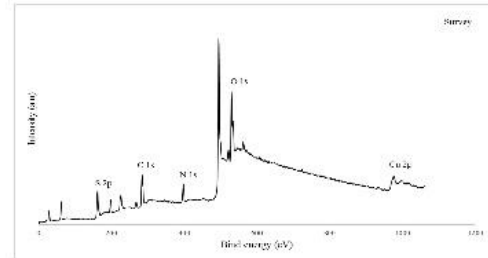

Figure 4. The survey XPS spectrum of BSA-capped CuNCs

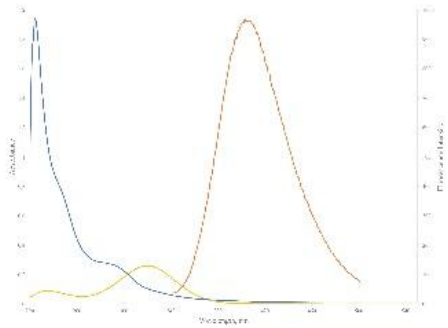

Figure 5. UV–vis absorption (blue line) and fluorescence emission (red line) spectra of CuNCs. absorption spectra of ORN (yellow line).

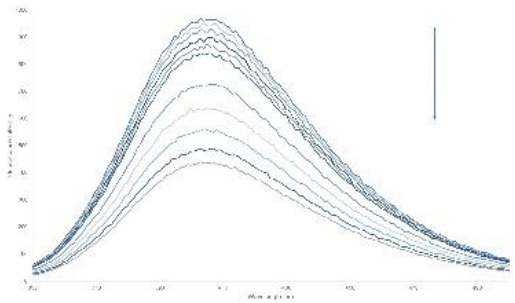

Figure 6. ORN concentration dependent fluorescence emission of the CuNCs.

Table 1. Regression and validation parameters Ornidazole by the CuNCs as a fluorescent probe

Linearity range (µg mL^{-1})	0,52-13,56
Slope	0,095
Intercept	0,9112
Correlation coefficient	0,9934
SE of slope	0,0112
SE of intercept	0,0206
LOD (µg mL^{-1})	0,0283
LOQ (µg mL^{-1})	0,0943
Intra-day precision (RSD%)	0,4591
Inter-day precision (RSD%)	0,6535

Table 2. Dosage form analysis of Ornidazole

Tablet (%)	Ampoule (%)
99,70	101,06
103,40	100,24
96,95	100,56
95,51	100,64
99,63	100,64
Mean	Mean
99,04	100,63
RSD %	RSD %
3,05	0,29

Table 3. Recovery analysis of Ornidazole by fluorescence spectroscopy

Added (µg mL^{-1})	Found (µg mL^{-1})	Recovery (%)
1.65	1.69	102,42
3,3	3.25	98,50
4,95	4.90	99,00

DEVELOPMENT OF FABRİC PHASE SORPTIVE EXTRACTION METHOD FOR DETERMINATION OF AZINPHOS-METHYL AND CHLORFENVINFOS PESTICIDES BEFORE HPLC-DAD ANALYSİS

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JUNE 22-25, 2021 ANKARA, TURKEY

13th International Symposium on Pharmaceutical Sciences

Introduction

Nowadays, fierce competition for the production of high quality and healthy agricultural products is a commonly discussed issue. Consequently, farmers use high amounts of pesticides to protect their products against pests and plant diseases. However, the usage of these compounds can result in harmful damages to the environment and may cause many human diseases such as Parkinson's disease, leukemia, asthma, and several types of cancer [1-3].

Materials and Methods

The influence of some important factors on the extraction efficiency of azinphos-methyl and chlorfenvinfos was optimized as follows: volume of sample for each fabric phase, 35 mL; kind of salt, Na_2SO_4; concentration of salt, 5%, *w/v*; rotating time in adsorption step, 35 min; kind of elution solvent, methanol; elution solvent volume: 800 µL; pH, 6; and vortex time, 2 min. Moreover, fabric phase sorptive membrane was characterized by scanning electron microscopy and fourier transform infrared spectroscopy.

Results

Fabric phase sorptive extraction was developed as an efficient, simple, and reliable method for the extraction of azinphos-methyl and chlorfenvinfos residues before their analysis with high-performance liquid chromatography combined with photodiode array detector.

Conclusions

Ease of operation, high values of EF, suitable RSDs, and low LODs and LOQs are the main advantages of the current method. Finally, fabric phase sorptive extraction was performed on the real samples and its efficiency for adsorption of the analytes from complex matrices has been successfully proved.

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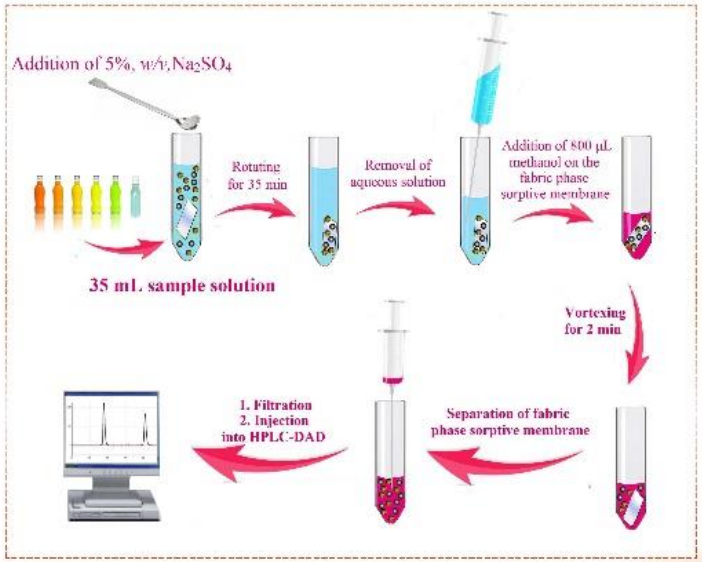

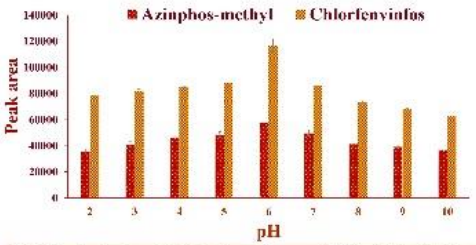

Table. Study of matrix effect and calculation of mean relative recoveries. 30, 50, and 300 µg L^{-1} of each pesticide were spiked into deionized water and real samples.

Mean relative recovery ± standard deviation (n=3)						
Analyte	Waste water	Apple juice	Orange juice	Carrot juice	Peach juice	Apricot juice
All samples were spiked with each analyte at a concentration of 30 µg L^{-1}						
Azinphos-methyl	87 ± 3	95 ± 4	89 ± 4	90 ± 5	93 ± 4	87 ± 5
Chlorfenvinfos	86 ± 4	90 ± 4	88 ± 5	88 ± 5	92 ± 4	88 ± 4
All samples were spiked with each analyte at a concentration of 50 µg L^{-1}						
Azinphos-methyl	92 ± 5	94 ± 3	93 ± 4	92 ± 4	94 ± 4	90 ± 4
Chlorfenvinfos	95 ± 4	93 ± 4	91 ± 5	92 ± 3	92 ± 3	91 ± 2
All samples were spiked with each analyte at a concentration of 100 µg L^{-1}						
Azinphos-methyl	93 ± 4	95 ± 3	94 ± 3	95 ± 4	95 ± 2	92 ± 4
Chlorfenvinfos	95 ± 3	96 ± 3	93 ± 4	90 ± 5	94 ± 5	93 ± 4

DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR THE DETERMINATION OF IMIDUREA IN CREAM FORMULATION

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JUNE 22-25, 2021 ANKARA, TURKEY

ISOPS 13th International SYMPOSIUM ON PHARMACEUTICAL SCIENCES

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Introduction: Imidurea is one of the substance that used as a preservative in pharmaceutical preparations and cosmetic products to prevent microbial growth (1-3). In this study, simple, selective and fast high performance liquid chromatographic method has been developed and validated for the analysis of imidurea that used as anti-microbial agent in cream formulation.

Materials and Methods: The chromatographic separation was carried out on CN (250x4.6 mm; 5 µm) column by using as mobile phase acetonitrile: water (25:75, v/v). The mobile phase flow rate was 1.0 mL/min. Imidurea was detected at 210 nm. The method was validated for system suitability, specificity, linearity, limit of quantification, limit of detection, robustness, recovery, precision and accuracy.

Extraction procedure of cream formulation

5.0 g cream sample

↓

Approx. 50 mL mobile phase (completed its volume in a volumetric flask)

↓

Vortex mixing (1 minutes) and ultrasonic bath at 30 min

↓

Filtered 0.45 µm nylon filter and 50 µL injection on HPLC system

Results: The calibration curve showed a linearity at 0.050-0.150 mg/mL range. The limits of detection and quantification were found to be 62.5 ng/mL and 125.0 ng/mL, respectively. Assay recovery of imidurea from cream formulation at 0.050, 0.100 and 0.125 mg/mL concentrations were evaluated (Table 1). Intra-day and inter-day relative standard deviation values were calculated to be less than 0.900%. The mean recovery was calculated as 101.86%.

Table 1: Recovery results for the assay of IMU

Concentration (mg mL^{-1})		Recovery (%)	RSD (%)
Added	Found (mean ± SD)		
0.050	0.050±0.001	98.857	0.744
0.100	0.104±0.001	104.560	0.363
0.125	0.128±0.001	102.153	0.363

Conclusions: The validated method was successfully applied to the determination of imidurea in cream formulation (Figure 1). The developed method is simple, fast, selective, reproducible and reliable can be used safely routine determination of imidurea in pharmaceutical preparations and cosmetic products.

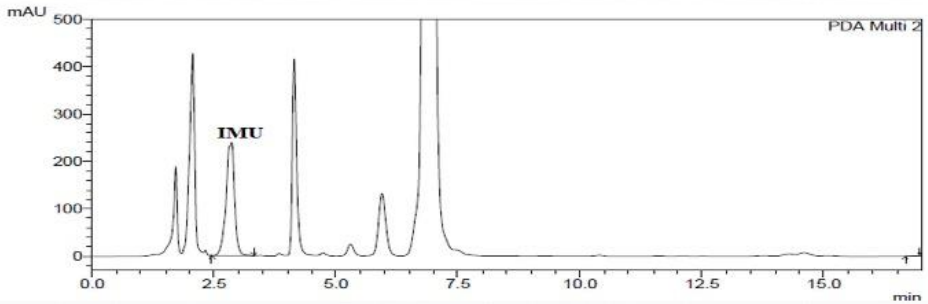

Figure 1: Chromatogram of imidurea (IMU) extracted from cream formulation (0.100 mg/mL)

References:

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A GQDS@PEDOT NPS-BASED ELECTROCHEMICAL TYROSINASE ENZYME BIOSENSOR FOR ADRENALINE DETECTION

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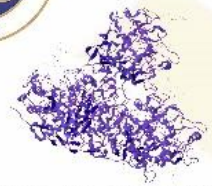

Introduction: An enzyme biosensor is a powerful analytical tool in which an enzyme as the biological sensing element is used to bind analyte(s) and a transducer for converting the recognition event into a measurable signal. Therefore, enzyme-based biosensors have great applications in drug and food analyses nowadays. Recently, graphene quantum dots (GQDs), with their versatile electrochemical and physical properties have attracted many attentions. In addition, poly(3,4-ethylenedioxythiophene) nanoparticles (PEDOT NPs) is a conducting polymer that has received enormous attention due to its high conductivity, good transparency, and stability. Therefore, these materials with such unique properties can be used for designing advanced electrochemical biosensors [1]. In this study, an electrochemical enzymatic biosensor is proposed for the detection of adrenaline. The biosensor design is achieved through immobilization of Tyrosinase (Tyr) in GQDS@PEDOT NPS platform on screen-printed electrodes (SPEs).

Materials and Methods: The surface of SPEs was activated with 0.1 M H_2SO_4 for 120 s at 3 mA using the chronopotentiometric method before each electrode modification. For electrode modification as a first step, GQDs nanomaterial suspension was dropped onto the working surface of the SPE and allowed to dry at room temperature. PEDOT NPs were dropped onto SPE/GQDs surface and allowed to dry at room temperature in the next step. In the final step, the Tyr enzyme was dropped on the surface of SPE/GQDs@PEDOT NPs. Tyr was immediately immobilized by crosslinking the nanobiosensor surface by adding a 0.25% GA crosslinking agent. Chronoamperometric (CA) determination of adrenaline was performed as follows: the prepared nanobiosensor platform was placed in an analytical cell containing 10 mL PBS with KCl (50 mM pH 6.5). 1.0 mM of the adrenaline was added after achieving steady-state current under stirring conditions within a working potential of −0.2 V at 300 rpm.

Results and Discussion:

Electrochemical characterization of the SPE/GQDs@PEDOT NPs/Tyr nanobiosensor

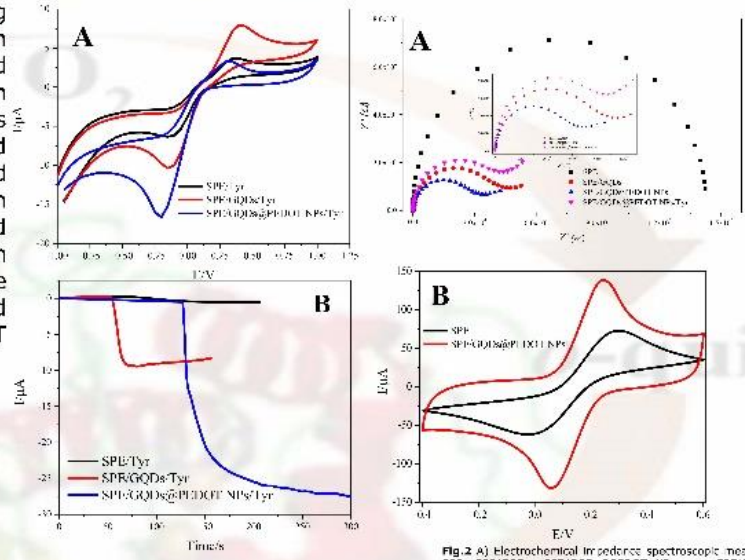

Fig.1 Cyclic voltammograms of SPE/Tyr, SPE/QDs/Tyr, and SPE/GQDs@PEDOT NPs/Tyr in the presence of catechol in 50 mM phosphate buffer at pH 6.5 with 0.1 M KCl. B) Current-time responses of SPE/Tyr, SPE/GQDs/Tyr and SPE/GQDs@PEDOT NPs/Tyr for the successive addition of 1 mM catechol solution, during 300 rpm stirring conditions within a working potential of -0.2 V using 50 mM phosphate buffer at pH 6.5 with 0.1 M KCl.

Fig.2 A) Electrochemical impedance spectroscopic measurements of SPE, SPE/GQDs, SPE/GQDs@PEDOT NPs, and SPE/GQDs@PEDOT NPs/Tyr. B) Cyclic voltammograms of SPE, and SPE/GQDs@PEDOT NPs in 5 mM $[Fe(CN)_6]^{3-/4-}$ with 0.1 M KCl as a redox probe.

Optimization of the biosensing response and analytical characterization of SPE/GQDs@PEDOT NPs/Tyr nanobiosensor

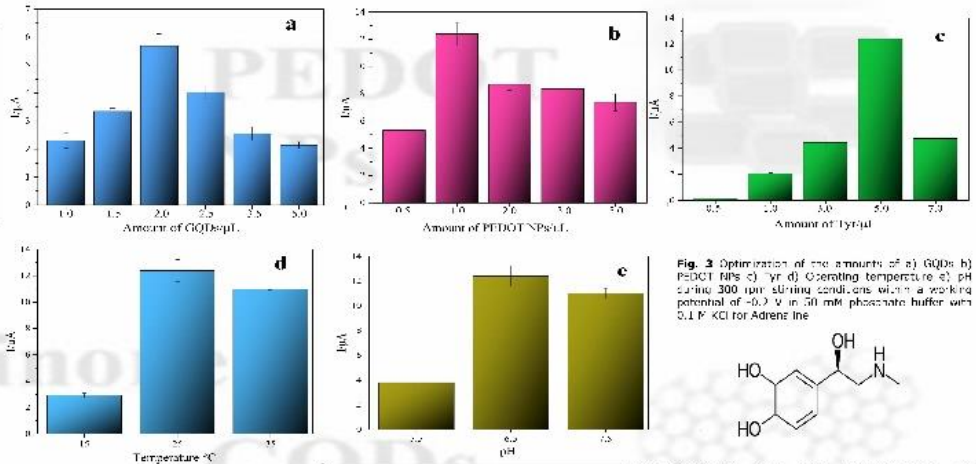

Fig. 3 Optimization of the amounts of a) GQDs b) PEDOT NPs c) Tyr d) Operating temperature e) pH during 300 rpm stirring conditions with a working potential of -0.2 V in 50 mM phosphate buffer with 0.1 M KCl for Adrenaline

Fig. 4 Typical current-time response curves for the successive additions of adrenaline. Inset: Analytical calibration of the adrenaline.

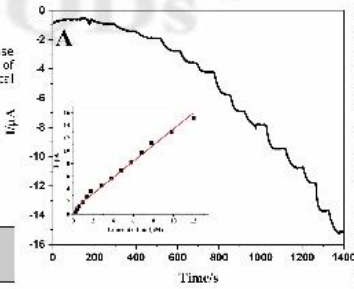

Table 1. Regression data for multi detection of adrenaline using SPE/GQDs@PEDOT NPs/Tyr nanobiosensor

Regression data	Adrenaline
Linearity range (µM)	0.2-12
Slope (µA.M^{-1})	1.27
Intercept (µA)	0.73
SE of slope	0.03
SE of intercept	0.18
Correlation coefficient	0.992
Limit of Detection (µM)	0.065
Limit of Quantification (µM)	0.2
Within-day precision[a] (RSD %)*	5.71
Between-day precision[a] (RSD %)*	9.28

Table 2. Detection of adrenaline from pharmaceutical dosage form

Parameters	Penepin® Auto-Injector
Labeled claim (mg/mL)	0.150/0.3
Amount found (mg/mL)	0.151/0.3
RSD%	1.94
Bias%	0.67
Amount added (µM)	5.00
Found (µM)	5.66
Average recovered %	106.00
RSD% of recovery	1.52
Bias%	-6.00

Conclusions: For the determination of adrenaline, CA response of SPE/GQDs@PEDOT NPs/Tyr nanobiosensor were followed by continuous addition of adrenaline with different and increased concentrations under optimized conditions. The linear range was achieved in the range 0.2 12µM for adrenaline. Limit of detection (LOD) and limit of quantification (LOQ) based on 3s/m and 10s/m principles, respectively, were calculated using a linear curve; where 's' is the standard deviation of the peak current of the lowest concentration of the adrenaline and 'm' is the slope of the related calibration curve. LOD and LOQ values were found as 0.065 µM, and 0.2 µM for adrenaline, respectively, using SPE/GQD@PEDOT NPs/Tyr nanobiosensor. In summary, an amperometric electrochemical nanobiosensor based on Tyr, GQDs, and PEDOT NPs has been developed for the first time for adrenaline detection. The proposed nanobiosensor offers a wide linear response range for adrenaline. Furthermore, the optimized nanobiosensor exhibited high sensitivity and low detection limit, with good selectivity and stability for detecting adrenaline in a pharmaceutical dosage form.

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SIMULTANEOUS QUANTITATION OF SULFUR METABOLITES IN CELL EXTRACT BY LC-MS/MS

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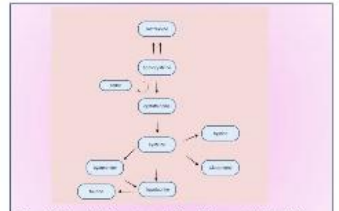
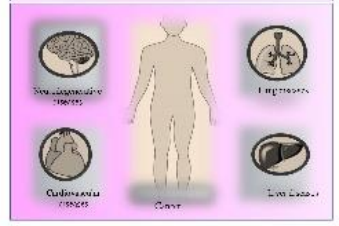
INTRODUCTION

Sulfur-containing metabolites play critical roles in cellular function. Changes in the levels and metabolism of sulfur compounds have been associated with various disorders such as cancer, neurodegenerative diseases, cardiovascular diseases, liver diseases and diabetes. The quantification of sulfur-related metabolites is essential for monitoring and diagnosing patients with disorders (1, 2). The main separation method used for the sulfur-containing metabolites from biological samples was reverse phase chromatography using C18 columns. However, these methods require a derivatization step to separate these polar metabolites (3). In this study, a new method is presented for the simultaneous analysis of metabolites in sulfur metabolism, which has a short analysis time and does not require a derivatization step.



Simplified metabolic network for sulfure metabolites (4)



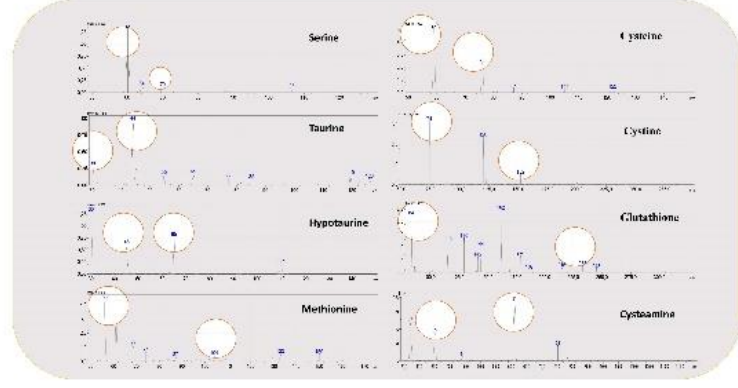
METHODS

HK-2 cell lines were used. After applying the necessary steps, all cells were isolated and 6 ml of solution belonging to each sample was obtained. The solutions were vortexed for 1 minute by adding 6 mL of cold methanol and 2 mL of cold chloroform, respectively. The samples were then shaken for 20 minutes at 800 rpm and then centrifuged at 4 °C for 5 minutes at 15000 rpm. After the centrifugation process, 3 separate phases were obtained. The upper phase (water and methanol phase) was used for this analysis.

For these prepared control groups and patient groups, 1 mL was taken from the upper phase solutions and the samples were left to evaporate. The volatized samples were diluted with 200 µL of mobile phase solution.

Chromatographic Parameters
Column: ZIC-HILIC (100x4.6mm, 5 µm)
Equipment: Shimadzu LC 20 AXR system
Mobile phase: A: % 0.1 formic acid B: % 0.1 formic acid in acetonitrile
Flow rate: 0.35 ml /min
Injection volume: 20 µl
Analysis time: 12 min
Column temperature: 30 °C

Equipment: : Shimadzu 8030 MS/MS
Scanning Speed: 2 Spectra/sn
Spray Voltage: 3500 (-V)
DL Temperature: 200 °C
Ion Source Temperature: 400 °C
Gas flow rate: 13 L/dk
Nebulizer 3 L/dk



DISCUSSION

We tested different chromatographic conditions including columns, mobile phases gradient elution programs for the separation of sulfur metabolites.

Columns
C18 (100x4.6 mm, 3 µm)
Amine (100x2 mm, 3 µm)
ZIC-pHILIC (50x2.1mm, 3 µm)
ZIC-HILIC: (100x4.6mm, 5 µm)

Column temperature (°C)
30
35
40

Mobile phases
A: % 0.1 formic acid
B: % 0.1 formic acid in acetonitrile
A: 20 mM ammonium acetate buffer
B: 0.1 formic acid in acetonitrile
A: 20 mM ammonium format buffer
B:0.1 formic acid in acetonitrile
A: 0.1 formic acid in %5 acetonitrile
B: 0.1 formic acid in %95 acetonitrile

Flow rate (mL/min)
0.4
0.35
0.3

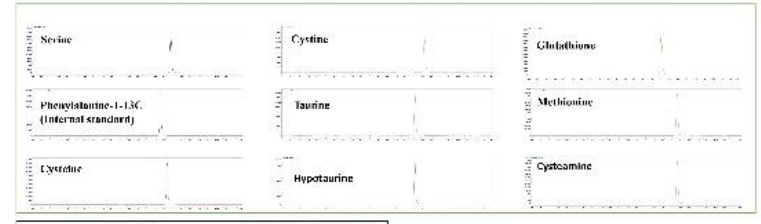
Run time (min)
12
15
17

Gradient elution (A: % 0.1 formic acid ,B: % 0.1 formic acid in acetonitrile)
1 min 95% B, 5 min: 5% B, 6 min 5% B, 8 min 5% B, 9 min 95% B, 12 min
1 min 90% B, 5 min 10% B, 6 min 10% B, 10 min 5% B, 9 min 90% B, 12 min
1 min 80% B, 5 min: 20% B, 6 min 20% B, 8 min 20% B, 9 min 80% B, 12 min

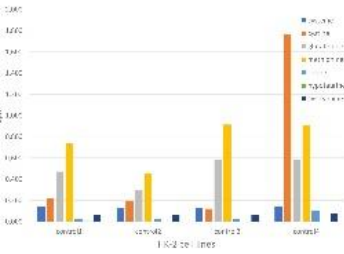
Effect of ionic strength
A: % 0.05 formic acid
B: %0,05 formic acid in acetonitrile
A: % 0.1 formic acid
B: %0.1 formic acid in acetonitrile
A: % 0.2 formic acid
B: % 0.2 formic acid in acetonitrile

RESULTS

A simple, specific, rapid and sensitive LC-MS/MS method has developed for quantitative analysis of sulfur contains metabolites (cystine, cysteine, methionine, glutathione, cysteamine, taurine, hypotaurine and serine). The chromatographic separation was carried out on a ZIC®-HILIC (100 x 4.6mm, 5 µm) column with the mobile phase composed of 0.1 % formic acid and 0.1 % formic acid in acetonitrile in gradient elution. MS/MS conditions were optimized by injection of 1 ppm of each metabolite in order to increase sensitivity. Quantification was performed using multiple reaction monitoring mode.
Several chromatographic conditions with different stationary phases (C18, amino and HILIC) were tested in order to obtain a suitable separation with short analysis time. The best separation was obtained with HILIC column.



Standart	Precursor ion > product ion (m/z)
Serine	106,0→60,1
	106,0→70,0
Methionine	149,8→56,05
	149,8→104,05
Cysteamine	77,8→61,0
	77,8→35,2
Cystine	241,0→152,1
	242,0→74,0
Cysteine	121,80→59,10
	121,80→76,20
Glutathione	308,1→233,1
	308,1→84,1
Hypotaurine	110,00→45,10
	110,00→65,10
Taurine	126,10→44,05
	126,00→80,10



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DETERMINATION OF SULFACETAMIDE, BETAMETHASONE, METHYL PARABEN AND PROPYL PARABEN IN PHARMACEUTICAL EYE DROP USING RP- HPLC

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Introduction

Sulfacetamide (SFS) is a sulfonamide group antibiotic. Betamethasone (BTM) is a corticosteroid . Methyl Paraben (MP) and Propyl Paraben (PP) are preservatives. These four compounds are pharmacologically active constituents found in pharmaceutical eye drop. There have been numerous publications describing various methods for the quantification of these compounds individually and in combination with other drugs[1,2,3]. However, literature survey reveals that no method has been reported for determination of SFS, BTM, MP and PP, simultaneously.

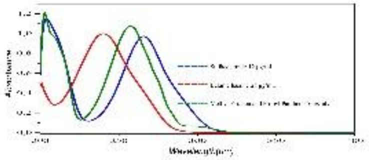
This study involves the development of reversed-phased chromatographic method for simultaneous determination of SFS, BTM, MP and PP present in a pharmaceutical eye drop.

Material and Method

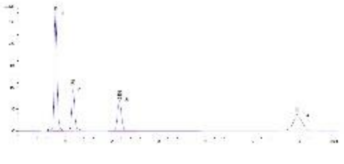
Stock solutions of SFS, BTM, MP and PP were prepared in methanol. Standard solutions were prepared from stock solutions by dilution with mobile phase. 50 µL of eye drop was accurately weighed and diluted to 50.0 mL with methanol. 100 µL of this solution was diluted water-asetonitrile(55:45). UV spectrophotometer (UV- 2450) was used for all measurements. The separation and detection of the analytes were carried out using an Agilent 1220 Infinity LC system equipped with UV detector. Spectra and chromatograms are shown in Figures.

HPLC parameters	
Column	Agilent Zorbax Eclipse XDB C18 (3.0x75mm, 3.5µm)
Dedector Wavelenght	250 nm
Column temperature	25°C
Mobile phase	A: Water B: Acetonitrile
Flow Rate	0.5 mL/min
Injection Volume	5 µL

Results



UV spectra of SFS, BTM and MP-PP



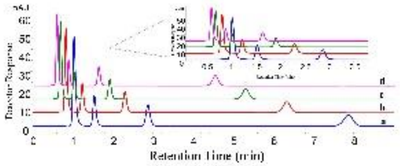
HPLC Chromatogram of 1 SFS, 2 MP 3. PP and 4 BTM

Analytic Parameters of RP - HPLC Method

Parameter	SFS	BTM	MP	PP
Linearity (µg/mL)	3.0-7.0	1.5-3.5	0.6-1.4	0.6-1.4
Correlation Coefficient r²	0.9984	0.9999	0.999	0.9991
Regression equation	y=20.357x- 3.3433	y=17.06x- 3.67	y=38.217x- 3.88	y=39.133x- 3.7933
Intraday precision (n=6) (% RSD)	0.78	0.54	0.89	0.79
Interday precision (n=3) (% RSD)	2.05	1.42	2.21	1.73
LOD (µg/mL)	0.37	0.33	0.021	0.012
LOQ (µg/mL)	1.126	1.015	0.064	0.035
Recovery	95,75	101.9	103.4	98.12

Effect of Flow Rate

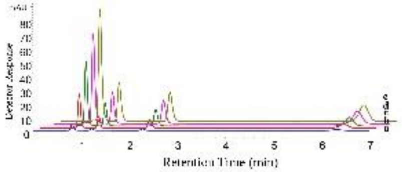
Flow rate (mL/min)		0.4	0.5	0.6	0.7
SFS	$t_{R(min)}$	1.032	0.321	0.681	0.583
	R	[illegible]	[illegible]	[illegible]	[illegible]
	N	[illegible]	2271	1954.8	1677.7
	Pas	1.402	[illegible]	1.31	1.259
BTM	$t_{R(min)}$	7.874	6.328	5.298	4.53
	N	14089.1	13771	13042.0	11988.2
	Pas	0.979	0.926	0.935	0.911
MP	$t_{R(min)}$	1.541	[illegible]	1.019	0.871
	R	7.999	7.559	7.165	6.605
	N	[illegible]	[illegible]	[illegible]	[illegible]
	Pas	1.32	[illegible]	1.268	1.225
PP	$t_{R(min)}$	2.8[illegible]	2.294	1.908	1.626
	R	15.68	15.503	15.014	14.44
	N	9868.6	9356.0	8452.2	7651.0
	Pas	1.134	1.118	1.119	1.113



Temperature: 25 °C, flow rate(mL/min): (a) 0.4, (b) 0.5, (c) 0.6, (d) 0.7 wavelength: 250 nm, injection volume: 10 µL, mobile phase: water: acetonitrile (v/v: 55:45)

Effect of Injection Volume

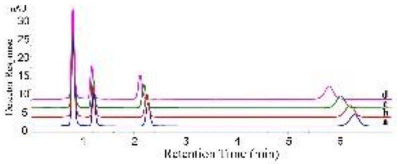
Injection Volume(µL)		1	5	10	15	20
SFS	$t_{R(min)}$	0.609	[illegible]	0.616	[illegible]	[illegible]
	R	3.854	3.726	[illegible]	3.273	3.025
	N	2786	[illegible]	2271.73	1894.3	[illegible]
	Pas	1.178	1.182	1.242	1.270	1.34
BTM	$t_{R(min)}$	5.521	[illegible]	[illegible]	[illegible]	[illegible]
	N	12904.4	[illegible]	[illegible]	[illegible]	[illegible]
	Pas	0.932	0.922	0.957	0.924	0.969
MP	$t_{R(min)}$	1.216	1.222	1.219	1.229	1.220
	R	8.304	[illegible]	7.669	7.339	[illegible]
	N	5895.3	[illegible]	[illegible]	[illegible]	[illegible]
	Pas	1.168	[illegible]	1.209	[illegible]	[illegible]
PP	$t_{R(min)}$	2.228	[illegible]	[illegible]	[illegible]	[illegible]
	R	16.261	16.229	16.078	[illegible]	[illegible]
	N	10168.6	10966.6	[illegible]	9161.2	[illegible]
	Pas	1.036	1.051	1.091	1.140	1.197



Temperature: 25 °C, flow rate: 0.5 (mL/min), wavelength: 250 nm, injection volume (µL): (a) 1.0, (b) 5.0, (c) 10.0, (d) 15.0, (e) 20.0, mobile phase: water: acetonitrile (v/v: 55:45).

Effect of Column Temperature

Temperature (°C)		25	30	35	40
SFS	$t_{R(min)}$	0.813	0.81	0.802	0.790
	R	3.723	3.68[illegible]	3.563	3.449
	N	[illegible]	[illegible]	[illegible]	[illegible]
	Pas	1.194	1.191	1.195	1.198
BTM	$t_{R(min)}$	6.291	6.197	6.011	5.779
	N	15275.6	15611.2	15962.8	16202.6
	Pas	0.931	0.947	0.956	0.962
MP	$t_{R(min)}$	1.217	1.208	1.184	1.159
	R	8.211	8.115	7.907	7.646
	N	5430.7	5408.9	5230.8	5100.3
	Pas	1.156	1.140	1.169	1.165
PP	$t_{R(min)}$	2.275	2.244	2.186	2.100
	R	16.509	16.649	16.541	16.554
	N	10989.2	10928.6	10607.3	10489.7
	Pas	1.057	1.069	1.083	1.097



Temperature (°C): (a) 25.0, (b) 30.0, (c) 35.0, (d) 40.0, flow rate: 0.5 (mL/min), wavelength: 250 nm, injection volume: 5.0 µL mobile phase: water: acetonitrile (v/v: 55:45).

Conclusions

In this study, RP-HPLC method is presented for the determination of SFS, BTM, MP and PP in eye drop which offers numerous advantages, such as good resolution, accuracy, precision, selectivity and ease of operation.

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Acknowledgement

This work was produced as a part of Onur DEMIR's MSC thesis

Voltammetric studies on the antibiotic drug cefprozil using a glassy carbon electrode

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Introduction

Cefprozil (CF) is an orally active, cephalosporin antibiotic. The range of antimicrobial activity of cefprozil is very broad (1). It is effective against gram-positive organisms and some gram-negative bacteria, including *Haemophilius influenzae*, *Moraxella catarrhalis*, *Escherichia coli*, *Klebsiella spp.* Cefprozil is used in the treatment of otitis media, upper and lower respiratory infections, and uncomplicated skin infections (2). In this study, cefprozil was investigated on a glassy carbon electrode with voltammetric methods.

Materials and Methods

All electrochemical experiments were performed using a Autolab Type II potentiostat/galvanostat with GPES 4.9 software. A three-electrode system was employed including a glassy carbon (GC) working electrode, a platinum wire counter electrode, an Ag/AgCl reference electrode. Stock solutions of cefprozil (1.0×10^{-3} M) were prepared in ultra-pure water. Phosphate buffer (PB), Britton-Robinson buffer (BRB), and acetate buffer (AB) solutions were used as supporting electrolytes at different pH values.

Results

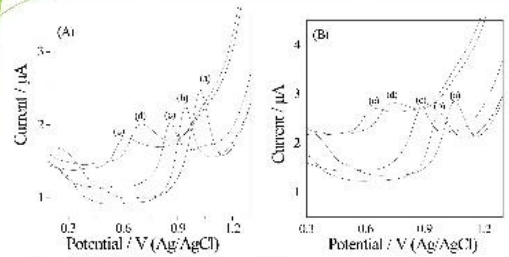

Fig. 1. (A) Differential pulse and (B) square wave voltammograms of 6×10^{-5} M CF in (a) PB at pH 2.0, (b) AB at pH 3.5, (c) BRB at pH 5.0, (d) PB at pH 7.0, and (e) BRB at pH 8.0

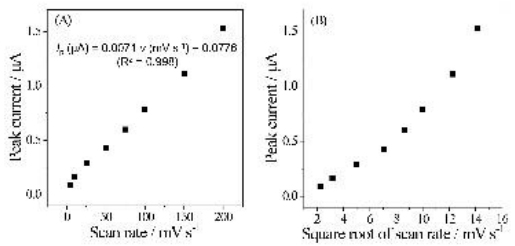

Fig. 3. Effects of scan rate on the peak current of 6×10^{-5} M CF in PB at pH 2.0 on GCE. Scan rates: 5, 10, 25, 50, 75, 100, 150 and 200 mV s^{-1}

Adsorption controlled process!
Deposition potential: 0.9 V , Deposition time: 45 s

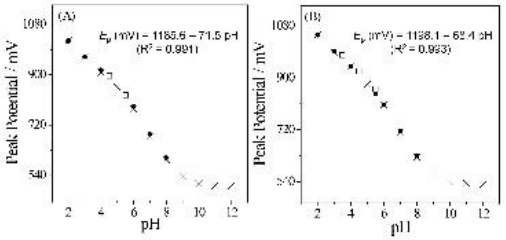

Fig. 2. Effects of pH on the anodic peak potential of 6×10^{-5} M CF in PB, AB, and BRB solutions at pHs between 2.0 and 12.0 obtained using (A) differential pulse voltammetry and (B) square wave voltammetry on GCE. ●: PB , ▫: AB, x: BRB

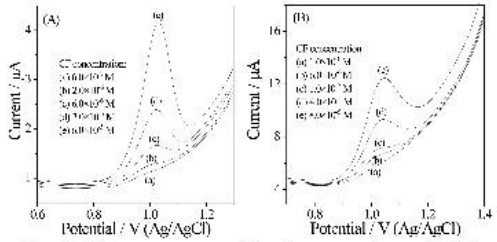

Fig. 4. Voltammograms at different concentrations of CF for (A) DPV and (B) SWV in PB at pH 2.0 on GCE

Table 1. Data of the calibration lines of CF in PB at pH 2.0 on GCE by DPSV and SWSV

Parameter	DPSV	SWSV
Peak potential (mV)	1028	1037
Linearity range (µM)	0.1 – 60	0.2 – 80
Slope (µA μM^{-1})	4.54×10^{-2}	7.22×10^{-2}
Intercept (µA)	1.06×10^{-1}	5.05×10^{-1}
Correlation coefficient, r^2	0.994	0.992
LOD (µM)	5.06×10^{-3}	1.12×10^{-2}
LOQ (µM)	1.53×10^{-2}	3.39×10^{-2}
Repeatibility of peak potential (RSD%)*	0.22	0.10
Repeatibility of peak current (RSD%)*	0.73	0.56
Reproducibility of peak potential (RSD%)*	0.41	0.20
Reproducibility of peak current (RSD%)*	1.49	1.39

* Concentration of CEF: 40 µM; Obtained from five experiments

Table 2. Results obtained from determination study of CF from Serozil® by DPSV and SWSV

Parameter	DPSV	SWSV
Labeled claim (mg)	500.00	500.00
Amount found (mg)*	500.21	500.32
RSD%	0.65	0.38
Bias%	0.042	0.034

* Obtained from five experiments

Table 3. Results obtained from recovery study of CF from Serozil® by DPSV and SWSV

Parameter	DPSV	SWSV
Added CF (g)	8.1488×10^{-5}	8.1488×10^{-5}
Found CF (g)*	8.1821×10^{-5}	8.1127×10^{-5}
Recovery%	100.41	99.56
RSD% of recovery	0.86	0.82
Bias%	0.41	-0.44

* Obtained from five experiments

Conclusions

We have described sensitive, simple, rapid, and selective voltammetric methods for the analysis of cefprozil in its pharmaceutical formulation.

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EFFECTIVENESS OF *ACHILLEA GONIOCEPHALA* LOADED NANOPARTICLE ENCAPSULATION ON ANTIOXIDANT AND CYTOTOXIC PROPERTIES

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JUNE 22-25, 2021 ANKARA, TURKEY
13th International Symposium on Pharmaceutical Sciences

Abstract

The present study aimed to prepare *A. goniocephala* chloroform extract, fractions, and evaluate antioxidant and cytotoxic effects. Then, the nanoparticles (NPs) were synthesized on the most efficient fraction in a certain systematic and the biological activities of the free forms of the extract/fractions were compared with the activities of the NP forms. Antioxidant capacities of 14 fractions obtained from chloroform extract by column chromatography were found by FRAP, DPPH and CUPRAC methods. Again, the cytotoxic activities of all fractions were evaluated on MCF-7 and HT29 cell lines using the XTT cell viability assay. Chitosan-tripolyphosphate (TPP) NPs were formed using the ionic gelation method of H and F extracts, which show the most active properties because of biological activities. The antioxidant and cytotoxic effects of the characterized NPs were also evaluated. The synthesized particle size of the NPs from two extracts obtained between 274.12 and 296.25 nm. The zeta potential values of NPs were between 2.3 and 3.0 mV. The extract-encapsulation and loading-efficiency of the most active NPs were 77.6± 0.04% and 7.76± 0.01% for F extract and 10.2± 0.02% and 1.39± 0.07% for H extract, respectively. Anticancer activity of chitosan NPs gave better results compared to unencapsulated extracts. As a result, it was found that the *A. goniocephala* extract can be encapsulated in chitosan NPs and has an antioxidant and more anticancer effect than the free forms.

Materials and Methods

The specimens of *A. goniocephala* were collected from field studies and determined by Prof. Dr. Turan Arabacı. A voucher specimen (T.Arabacı 2957) was deposited at the herbarium of the Faculty of Pharmacy, İnönü University, Malatya, Turkey for future reference.MCF-7 human breast cancer cells, and HT29 human colorectal adenocarcinoma cells were obtained from the American Type Culture Collection (ATCC). Dulbecco's modified Eagle's medium (DMEM) (ATCC, USA), phosphate buffer saline (PBS), and fetal bovine serum (FBS) were purchased from PAA Ltd. (France). Chitosan (400 kDa, DD 87) were obtained from Fluka. L-glutamine-penicillin-streptomycin solution were purchased from Sigma-Aldrich. XTT reagent (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) was purchased from Roche Diagnostic.

Extraction procedures: The aerial parts of the plant were dried in the shade (25°C) and ground to a fine powder in a mechanic grinder (Renas, RBT1250). The powdered samples were extracted with organic solvents (methanol, and chloroform) using the maceration method until colourless. The organic phase was evaporated to dryness under decreased pressure. All extracts were maintained at 4°C for future analysis.

The cytotoxic activities of chloroform (IC_{50} = 0.019 mg/mL) and methanol (IC_{50} = 20.391 mg/mL) extracts were assessed on the MCF-7 cell line, and it was observed that the chloroform extract was more potent against MCF-7 than methanol extract. In this study, column chromatography was performed to separate the possible effective compounds contained in the effective chloroform extract according to its polarity. The chloroform sub-extract (7 g) was inserted to a silica gel column and eluted with toluene, petroleum ether and methanol mixture.

Antioxidant assays: Plant samples were tested with Ferric Reducing Antioxidant Power (FRAP) assay. [1] The FRAP results were expressed as mM Fe^{2+}/mg extract. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity of the extracts was evaluated the experimental protocol found in the literature [2]. Results are expressed as IC_{50} values (mg/mL). In Cupric Reducing Antioxidant Capacity (CUPRAC) assay [3]. CUPRAC values were given as mM TroloxE/mg extract.

Cell culture conditions: Cell culture studies were performed using modified method of [4]. After the XTT results were evaluated, NPs of the two extracts showing the highest cytotoxicity in MCF7 and HT29 cells were prepared. NPs containing different amounts plant extract were applied to the cells separately, and the differences between their cytotoxic effects were evaluated by calculating the IC_{50} values. To calculate the IC_{50} value, samples and NPs containing 12.5, 25, 50, and 100 µg amount of plant extracts were treated with the cells.

Preparation of chitosan NPs containing chloroform extract and Measurement of EE and LC: NPs were prepared using ionic gelation method as stated [5]. Ultraviolet visible spectrophotometer was used to measure the encapsulation efficiency (EE %) and loading capacity (LC %) of the extract in NPs. A standard calibration curve of the extract was established at 340 nm. The amount of extract in the supernatant was calculated from the line equation obtained from this curve. The following equations were used to determine the encapsulation efficiency and loading capacity of the particles.

$$EE\ (\%) = \frac{m_o - m_s}{m_o} x100 \quad LC\ (\%) = \frac{m_o - m_s}{W_{np}} x100$$

where, mo is the initial mass of natural extracts and ms mass of natural extracts in the supernatant and wnp = total weight of the naturally obtained extract of NPs [6]. Total phenolic contents in the extracts and NPs were determined by Folin-Ciocalteu colorimetric method. The percentage of the encapsulated extract into chitosan NP was also determined by using a total phenolic assay according to the following equation [7].

$$EE\ (\%) = \frac{Actual\ phenolic\ of\ the\ extract\ entrapped\ in\ NPs}{total\ phenolic\ of\ initial\ extract} x100$$

Results

Antioxidant results of extracts and NPs

Table 1. Antioxidant capacity results of the fractions, reference and nanocapsules

Extracts/ compound	DPPH (IC_{50}: mg/mL)	CUPRAC (mM trolox /mg extract)	FRAP (mM $FeSO_4$ /mg extract)
A	0.611±0.218	0.240±0.073	9.79[illegible]±0.50[illegible]
B	0.561±0.077	0.541±0.015	8.750±0.181
C	1.052±0.22[illegible]	0.566±0.008	10.35±0.150
D	[illegible]178±0.280	0.116±0.075	[illegible]1.555±0.7[illegible]6
E	[illegible]335±0.356	0.7[illegible]2±0.018	10.[illegible]06±0.070
F	**0.895±0.062**	2.582±0.155	11.746±0.709
G	0.809±0.101	2.006±0.15[illegible]	13.540±0.354
H	**0.264±0.057**	1.111±0.058	10.879±0.152
I	[illegible]34[illegible]±0.635	1.050±0.010	10.990±0.244
J	[illegible]265±0.278	3.155±0.172	[illegible]1.565±0.2[illegible]8
K	0.890±0.289	0.[illegible]80±0.0[illegible]	10.395±0.16[illegible]
L	[illegible]5[illegible]7±0.210	0.70[illegible]±0.060	0.[illegible]6±0.09[illegible]
M	[illegible]017±0.688	0.564±0.093	10.[illegible]76±0.1[illegible]
N	[illegible]490±0.423	0.412±0.017	10.154±0.173
H capsule	[illegible]054±0.165	0.155±0.042	8.780±0.7[illegible]0
F capsule	[illegible]42[illegible]±0.060	0.759±0.040	8.69[illegible]±0.085
Ascorbic acid	0.0025±0.0004		
BHT		[illegible]462±0.074	21.7[illegible]6±0.776

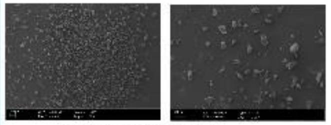


Fig. 1 SEM images of chitosan NPs

Characterization of NPs

Table 2 Zeta potential, particle size, and PDI values of NPs loading with extract

Formulations (Chitosan-TPP NPs)	Zeta potential(mV) ± SD	Size(nm) ± SD	PDI ± SD
*NP1	2.3 ± 0.01	274.12 ± 2.[illegible]	0.29[illegible] ± 0.03
*NP2	2.7 ± 0.02	290.15 ± 3.6	0.313 ± 0.03
*NP3	3.0 ± 0.01	296.25 ± 3.[illegible]	0.342 ± 0.04

* NP1, NP2, and NP3 containing chitosan (MW: 400 kDa), TPP and *A. goniocephala* chloroform extract

Table 3 Encapsulation efficiency and loading capacity of the NPs

Parameter	F extract	H extract
Linear equation±SD	y = 0.2808x+0.02339	y = 0.4667x-0.002409
Slope±SD	0.2808± 0.02	0.4667± 0.01
Intercept±SD	0.02339± 0.001	0.002409± 0.0007
r	0.9921	0.9925
EE %	77.6± 0.04	10.2± 0.02
LC %	7.76± 0.01	1.39± 0.07
TPC	0.0174± 0.0005	0.0566± 0.0010
NP	0.0112± 0.0003	0.0084± 0.0003
EE % (TPC)	64.37± 0.06	14.84± 0.11

Cytotoxicity results of extracts and NPs

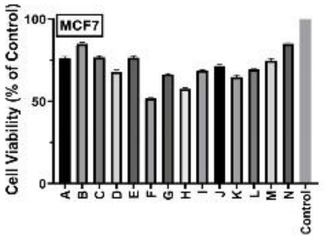


Fig. 2 Cell viability results of chloroform extracts of the plant. Cells were treated with samples at a concentration 50µg/mL. Cell viability of the control group was determined as 100%

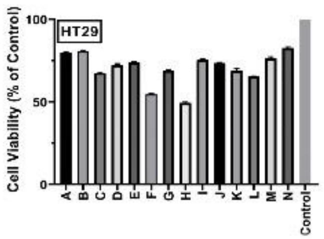


Fig. 3 Cell viability results of chloroform extract of the plant. Cells were treated with samples at a concentration 50µg/mL.

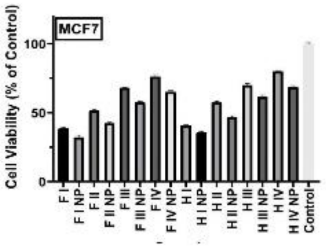
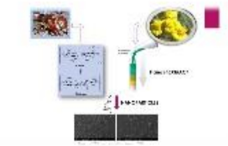


Fig. 4 Cell viability results of F and H chloroform extract and NPs. MCF7 cells were treated with samples at determined concentration (I 12.5 µg/mL, II 25 µg/mL, III 50 µg/mL and IV 100 µg/mL). Cell viability of the control group was determined as 100%



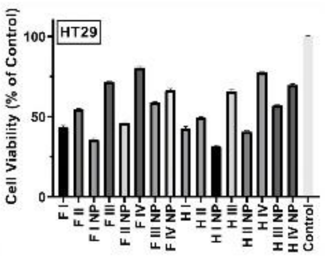


Fig. 5 Cell viability results of F and H chloroform extract and NPs. HT29 cells were treated with samples at determined concentration (I 12.5 µg/mL, II 25 µg/mL, III 50 µg/mL and IV 100 µg/mL). Cell viability of the control group was determined as 100%

Conclusions

According to the results of the XTT cytotoxicity and all the antioxidant assays study, among 14 extracts, F and H chloroform extracts of *A. goniocephala* showed better antioxidant cytotoxic, and especially anticancer activity. In addition, chitosan NPs containing these extracts have the higher anticancer effect is valuable for this study. Hence, anticancer activity of chitosan NPs gave better results compared to unencapsulated extracts. Based on these results, it can be said that the preparation of NPs containing the chloroform extract of *A. goniocephala*, cell culture studies of NPs containing the extract, and studies similar to this study will support future studies.

Acknowledgments

We would like to thank the Scientific and Technological Research Council of Turkey (TÜBİTAK) for the financial support of this research (Project No: 116S509) and Serkan KÖSTEKCİ for help during the field studies.

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DETERMINATION AND POSSIBLE MECHANISMS OF FORMATION LUMACAFTOR DEGRADATION PRODUCTS WITH USING LCMS-IT-TOF

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JUNE 22-25, 2021 ANKARA, TURKEY

ISOPS 13th International SYMPOSIUM ON PHARMACEUTICAL SCIENCES

ABSTRACT

The availability of high-resolution mass spectrometry (HRMS) in qualitative analysis provides the detectability and identifiability of the compounds in any matrix. HRMS has been very successful in explaining small molecule compounds such as suspicious and non-targeted degradation products, metabolites or pollutants in forensic medicine, drug discovery, metabolomic studies. There is no information about the degradation behavior of the active pharmaceutical compound lumacaftor when the previous studies were examined. In the current study, an LCMS-IT-TOF method was developed and used for forced degradation products of LUMA. Possible mechanism of production of degradation products were discussed and some new compounds were added to literature.

INTRODUCTION

By combining LC/IT-MS^n with LC/TOF-MS, a reliable methodology is provided by gaining accurate mass measurement, fragmentation data, and elemental characterization characteristics of the structure. The measurement of correct masses at 5 ppm is widely accepted for verification of mass determination. HRMS can provide information in m/z value in the 0.0001 digits. Thanks to the information obtained from the TOF analyzer, possible molecular formulas of the unknown compounds can be determined (1).

EXPERIMENTALS

Ion Trap-Time-of-Flight mass spectrometer (LCMS-IT-TOF) instrument was from Shimadzu (Japan); the whole system was consisted of DGU-20A3 degasser, LC-20AD gradient pump, SIL-20A autosampler, CTO-10ASVP column oven, CBM-20A communication module and IT-TOF mass spectrometer. LCMSsolutions version 3.80 software was used for data acquisition and evaluation.

The gradient mode has been redesigned as follows: 0th min 35% mobile phase A; 0.0–8.00 min gradient up to 90% mobile phase A; 8.01–10.0 min gradient continued 90% mobile phase A; 10.00–10.01 min gradient down to 35% mobile phase A; 10.01–12.00 min gradient continued 35% mobile phase A to condition the stationary phase to initial conditions. During the analysis, the mobile phase flow rate was 0.5 mL/min and the column temperature as 40.0 ± 0.1 °C. The autosampler temperature was fixed at 15±0.1 °C in order to maintain the stability of the sample and standard solutions, and the injection volume was determined as 10 µL. All mobile phase solutions prepared were filtered through 0.22 µm CA filter.

The forced degradation solutions were prepared according to ICH Q1A(R) stability testing of new drug substances and products guideline (2).

RESULTS

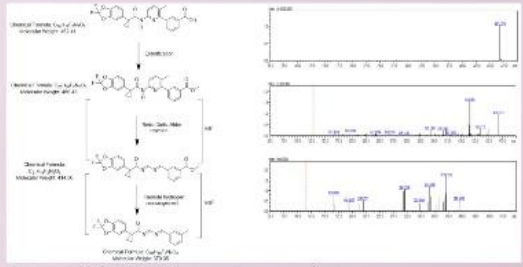

Figure 1. Possible formation mechanism and MS^n spectra of New DP2

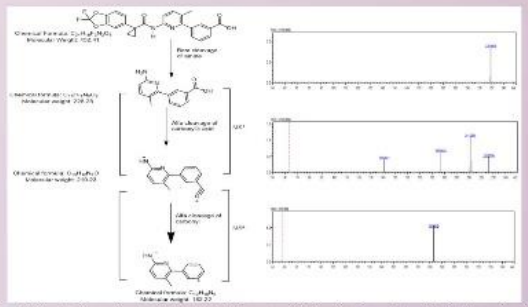

Figure 2. Possible formation mechanism and MS^n spectra of 3-[(6-amino-3-methylpyridin-2-yl)benzoic acid

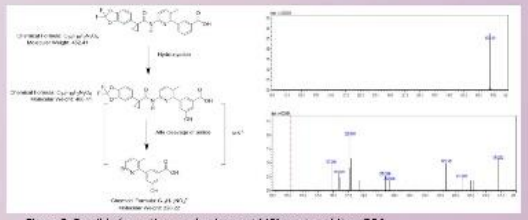

Figure 3. Possible formation mechanism and MS^n spectra of New DP4

RESULTS

Degradation products were obtained in a short time when the substance was exposed to various degradation conditions with heat at 60 °C. The decomposition products obtained are given in the Table 1 & 2. In acidic, basic and oxidative degradation conditions, 2, 2 and 3 compounds were obtained, respectively. Although the chromatograms were not presented here, it is also worth mentioning that when only temperature and light exposure it did not resulted in any degradation of LUMA; hence, there was no change in its amount observed. Identified compounds are given in Figures 1-3

Table 1. Retention times and maximum absorption wavelengths of the detected compounds.

Degradation Conditions	Compound	t_R (min)	λmax (nm)
	LUMA	7.9	216
Acidic	New DP1	6.2	271
	New DP2	9.8	223
Basic	3-[(6-amino-3-methylpyridin-2-yl)benzoic acid	3.5	324
	New DP1	6.2	271
Oxidative	New DP3	3.4	333
	New DP1	6.2	271
	New DP4	6.7	223

Table 2. Characterization of various forced degradation conditions of LUMA using HPLC-ESI-IT-TOF-MS in the positive ion mode.

Compound	Measured m/z $[M-H]^+$	Predicted m/z $[M-H]^+$	Error (ppm)	Score	Double-Bond Equivalent	Molecular Formula
LUMA	453.1235	453.1257	-4.86	100.0	16.0	$C_{24}H_{18}N_2O_5F_2$
New DP1	ND*	ND*	ND*	ND*	ND*	ND*
New DP2	467.1388	467.1413	-5.35	86.53	16.0	$C_{25}H_{20}N_2O_5F_2$
3-[(6-amino-3-methylpyridin-2-yl)benzoic acid	229.0969	229.0972	-1.31	93.97	9.0	$C_{13}H_{13}N_2O_2$
New DP1	ND*	ND*	ND*	ND*	ND*	ND*
New DP3	ND*	ND*	ND*	ND*	ND*	ND*
New DP1	ND*	ND*	ND*	ND*	ND*	ND*
New DP4	469.1199	469.1206	-1.49	76.20	16.0	$C_{24}H_{18}N_2O_6F_2$

*ND: Not detected

ACKNOWLEDGMENTS

This research was funded by Anadolu University Scientific Research Projects Fund Commission grant number 20055039.

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STABILITY-INDICATING RP-HPLC METHOD FOR ROBUST DETERMINATION OF LUMACAFTOR IN THE PRESENCE OF IVACAFTOR AND ANALYSIS OF ITS PHARMACEUTICAL FORMULATION

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Abstract

Cystic fibrosis is an inherited disorder caused by mutations in the gene encoding the cystic fibrosis transmembrane conductivity regulator (CFTR). Different mutations in the CFTR gene can cause cystic fibrosis, and the disease is divided into several sub-classes according to their effects on the CFTR protein. New drugs have been developed to treat gene dysfunction, and Lumacaftor (LUMA) is one of these drugs. It is used as a combination drug therapy with Ivacaftor (IVA). A novel stability-indicating HPLC methods have been developed for determination of LUMA in presence of IVA in bulk and pseudo tablet formulations, in this study.

Aim of the Study

The aim of this study is to develop a validated, reliable and easy-to-reproduce high-performance liquid chromatography method for the determination of LUMA in the presence of its degradation products and IVA, and its determination in its pharmaceutical formulation. The method validation was conducted in accordance with the International Council for Harmonization (ICH) guidelines.

Introduction

The U.S. Food and Drug Administration (FDA) approved LUMA/IVA as combination therapy on July 2, 2015 for patients aged 12 years and older who are homozygous for the F508del mutation (1). This combination has the property of being the first therapy to target and partially correct the primary defect in the F508del mutation. This consent was later updated for patients aged 6 and over in 2016 (2).

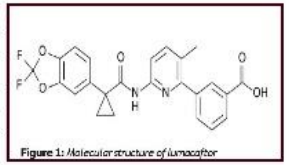

Figure 1: *Molecular structure of lumacaftor*

Based on this progress, Orkambi™, a fixed-dose tablet that combines LUMA and IVA, was developed by Vertex Pharmaceuticals. It is currently indicated for the treatment of cystic patients in various countries including the USA and EU countries. The recommended dose is two 200/125 mg tablets every 12 hours (3).

EXPERIMENTALS

Instruments

Nexera-i LC 2040C 3D device from Shimadzu (Japan) company was used for HPLC analysis.

Reagents & Materials

LUMA, used as standard substance, was purchased from TRC Company (Canada) with 99.9% (w /w) purity.

Standard Preparation

LUMA stock solutions were prepared by dissolving 5 mg of LUMA in methanol to obtain a solution at concentration of 200 µg/mL.

Chromatographic Conditions

Table 1. Gradient elution conditions.

Time (min)	Organic Phase (%)
0.00	30
0.00 - 8.00	30 → 90
8.00 - 8.01	90
8.01 - 9.00	90
9.00	Stop

Quantitation of LUMA, and its separation with IVA and other degradation products were achieved on an Ascentis® Express F_5-bonded fused-core silica particle column (2.7 µm particle size 100 × 4.6 mm, Supelco) using the mobile phase consisted of 0.1% (w/w) formic acid in water and 0.1% (w/w) formic acid in acetonitrile (pH~2.5). Gradient elution was performed at 1 mLmin^{-1} flow rate, according to the program given in Table 1. Samples were injected into the system as 10 µL aliquots and detecting the compounds at 216 nm. With the developed method, the pseudo formulation of Orkambi® was prepared and analyzed.

Results and Discussions

Table 2. System suitability values for LUMA

Parameter	Observed value	Acceptance Criteria
Retention time (min) ±CI*	5.31±0.006	-
Relative standard deviation(%) of retention time	0.33	RSD ≤ 1%
Precision for area (n=6)	0.36	RSD ≤ 1%
Injection precision for retention time (n=6)	0.05	RSS ≤ %1
Tailing factor (T)	1.06	T ≤ 2
Capacity factor (k)	5.48	2<k<10
Number of theoretical plates (N)	85400	N > 2000
USP Width	0.07	≤1
HETP (USP)	1.70	-

*Confidence interval at 95% confidence level

Table 3. Linearity and precision studies for LUMA

Parameter	Obtained value
Linearity range	0.5-20.0 (µg/mL)
Slope±SD* (intraday, n=10)	54118.23±581.24
Intercept±SD* (intraday, n=10)	-66798.90±8251.31
Regression coefficient (intraday, n=10)	0.997747
Limit of detection	200 ng/ml
Limit of quantitation	500 ng/mL
Slope±SD* (interday, n=30 x 3 days)	55005.98±1506.80
Intercept±SD* (interday, n=30 x 3 days)	69905.70±11682.83
Regression coefficient (interday, n=30 x 3 days)	0.996165
ANOVA	Ftt 2.71>F=0.001364 F=0.998637 (P>0.05)
Repeatability (intraday, mean, n=6)	8743420
Repeatability (intraday, SDr, n=6)	3133.40
Repeatability (interday, mean, n=18)	8758452.5
Repeatability (interday, SDr, n=18)	9091.16
ANOVA	F (2.35)=0.00 P=0.462641 (P>0.05)

* Standard deviation, * Standard error

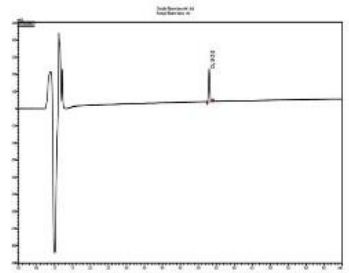

Figure 2: *Chromatogram of standard LUMA solution (C=10 µg/mL)*

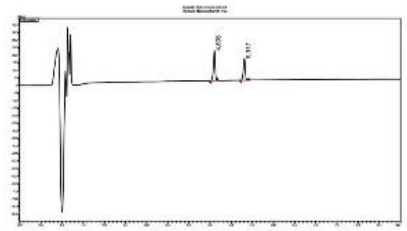

Figure 3: *Chromatogram of tablet Orkambi® solution (C_{LUMA}=10 µg/mL)*

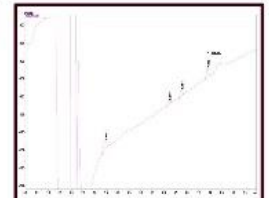

Figure 4: *The forced degradation chromatogram of oxidative stress (88 µg/mL)*

The substance was prepared in 15% (w/w) H_2O_2 solution and 3 compounds were obtained.

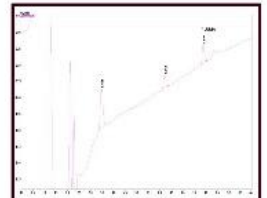

Figure 5: *The forced degradation chromatogram of basic stress (88 µg/mL)*

The substance was prepared in 1 N NaOH solution and 2 compounds were obtained.

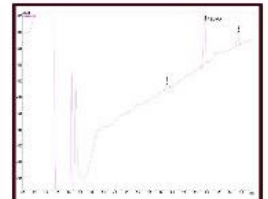

Figure 6: *The forced degradation chromatograms of acidic stress (88 µg/mL)*

The substance was prepared in 1 N HCl solution and 2 compounds were obtained.

According to the robustness studies, the retention time is affected the most by the organic component ratio of the mobile phase and the least by the wavelength change. In addition, the effect of formic acid concentration and the column temperature are below 1.5%. The parameter that is weakest to the applied changes is the peak area among the retention time, resolution, and tailing factor.

Table 4. Recovery studies

	Found	Precision		Accuracy	
Added (µg/mL)	(µg/mL)±CI*	SD	RSD (%)	Recovery (%)	Error (%)
8.00	8.01±0.11	0.11	1.41	100.17	+0.17
10.00	9.75±0.23	0.23	2.42	97.50	-2.50
12.00	11.56±0.41	0.42	3.64	96.30	-3.70

*Confidence interval at 95% confidence level

Table 5. Prepared Orkambi® formulation results (n=6)

	Obtained value
Mean (mg)	97.50
Standard deviation (mg)	0.80
RSD%	0.82
Standard error of mean (mg)	0.33
Bias %	2.51
CI*	±1.64

*Confidence interval at 95% confidence level

Conclusions

In summary, this is the first HPLC method, which was developed for analysis of LUMA in pharmaceutical formulation in the presence of IVA. During the method development phase, all validation and optimization parameters, which were suggested by international authorities were studied in detail.

Acknowledgments: This research was funded by Anadolu University Scientific Research Projects Fund Commission grant number 2005S039.

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SPRAY DRYER OPTIMIZATION OF TEA (*Camellia sinensis* L.) EXTRACT FROM DUST CHAMBER RESIDUES AND OVEN FIBERS COUPLED WITH ARTIFICIAL INTELLIGENCE

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Faculty of Pharmacy

INTRODUCTION

Tea is the second most commonly drunk liquid after water (1). The chemical components of tea leaves include polyphenols (catechins and flavonoids), alkaloids (caffeine, theobromine, theophylline, etc.), essential oils, polysaccharides, amino acids, lipids, vitamins (e.g., vitamin C), inorganic elements (e.g. aluminum, fluorine and manganese), etc. (2). Based on the established studies, classical regression tools have been widely used, but they have been generally associated with low accuracy levels, giving room to the development of the AI methods that are considered as accurate and non-linear intelligence tools (3).

MATERIALS and METHODS

In this study, dust chamber residues and oven fibers were extracted by soxhlet aparey with 11.5 % + 0.3 yields. This extract was powdered by a spray dryer under different conditions for optimization. Qualitative HPLC analysis of this extract showed that dust chamber residues and oven fibers contain catechin varieties. Normally, these residues are considered as waste, but as a result of this study, it is clear that these tea residues have the potential to be used as dietary supplements. This is the first study to use green tea residues obtained from the waste of commercial product production. The study equally employs the use of a non-linear model ANFIS and a traditional regression model MLR. From the AI result, it can be observed that both two models are capable of simulating the yield of extract from dust chamber residues and oven fibers using spray dryer optimization of tea.

RESULTS

As a result of the spray dryer experiments, 11.5% + 0.3 was the best yield obtained at 85-90oC temperature and 9 ml/min flow rate. From the AI result, it can be observed that both the two models are capable of simulating the yield of extract from dust chamber residues and oven fibers using spray dryer optimization of tea. The result further shows that ANFIS with R^2=0.9899 at the testing phase as a non-linear model has outperformed the classical regression model MLR having R^2=0.92017 and increases its performance accuracy up to 7% using the determination coefficient.

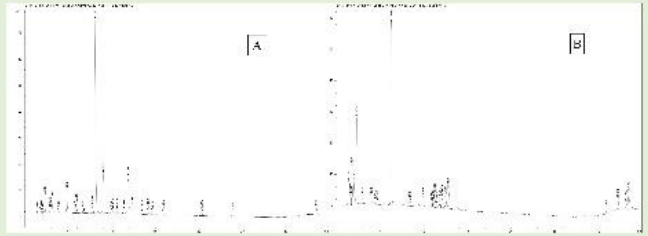


Figure 1. HPLC chromatograms of EGCG in (A) green tea standardized extraction and (B)

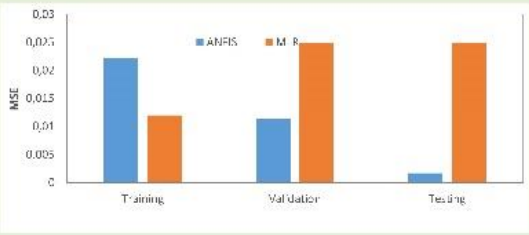
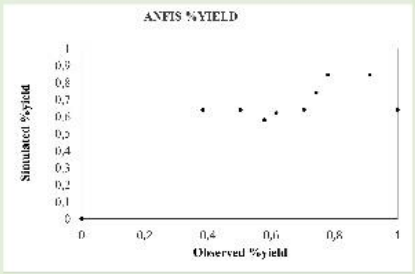


Figure 2. Bar chat showing the MSE values of ANFIS and MLR models for the training, validation and testing phases



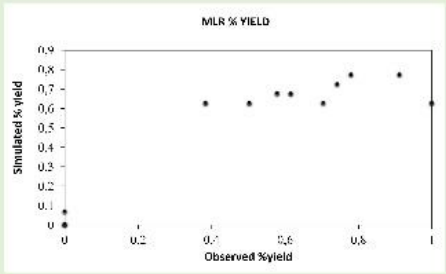


Figure 3. Scatter plots for ANFIS and MLR, of the %yield of the green tea extract

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DETERMINATION OF THYMOQUINONE FROM BLACK CUMIN USING HPLC TECHNIQUE: A CHEMOMETRICS BASED APPROACH

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INTRODUCTION

Natural compounds have been reported to show promising properties in the prevention of various diseases and disorders. Thymoquinone (TMQ) is the major ingredient found in black cumin and other medicinal plants (1). This bioactive ingredient has been reported in traditional Arab herbal medicine for curing different diseases (2). In this study, both simple and ensemble machine learning techniques were used in modelling both the qualitative and quantitative properties of TMQ using high-performance liquid chromatography (HPLC).

MATERIALS and METHODS

In this study, three artificial intelligence-based models were employed including two non-linear models, namely Hammerstein-Weiner (HW) and Neuro-fuzzy (NF) model and a classical linear model Multi-linear regression analysis (MLR) for the qualitative prediction of Thymoquinone (TQ) in HPLC optimization method development. Various parameters including the composition of the mobile phase, pH, flow rate and concentration of the standard are used as the independent variables, whereby the retention time of TQ is simulated as the dependent variable. The predicted and experimental results were further evaluated using two performance indices: determination coefficient (R2) as the goodness of fit and mean squared error (MSE).

RESULTS

The obtained results depicted the promising ability of the non-linear models NF and HW over MLR. For the ensemble machine learning technique, the results obtained from the non-linear ensemble method (Neuro-fuzzy ensemble (NF-E)) show its ability to boost the performance efficiency of the single models up to 28%. Additionally, NF-E proved its superiority over the linear ensemble technique (weighted average ensemble (WAE)) and increased its performance prediction up to 4.6% in the testing stage. The general results indicated the satisfactory and ruggedness of AI-based models as well as justified the boosting ability of the ensemble machine learning for the prediction of TQ.

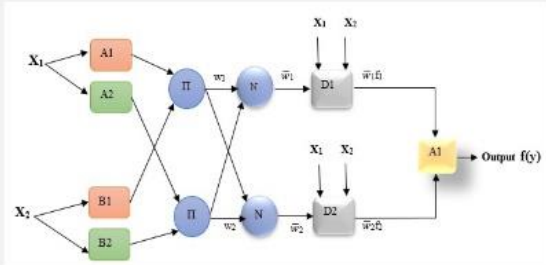

Figure 1. Architecture structure of Neuro-Fuzzy model

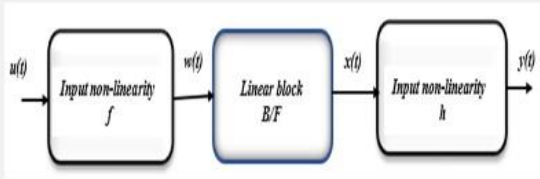

Figure 2. Schematic of the Hammerstein-Wiener model

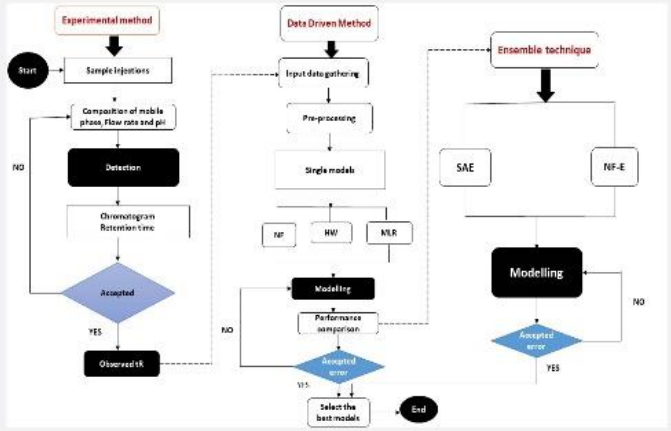

Figure 3. Proposed flowchart of experimental data-driven methods

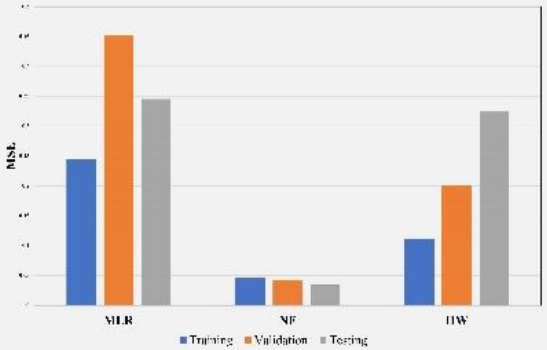

Figure 4. Comparison of performance error of the single models

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SIMULTANEOUS DETERMINATION OF A BINARY MIXTURE IN A DOSAGE FORM BY CHEMOMETRIC METHODS

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Introduction: In spectrophotometric studies, derivative spectrophotometric methods have been used for the quantitative resolving of binary mixtures. With the development of chemometric techniques many problems of the simultaneous analysis of two-component or multi-component mixtures have been solved (1–3). The multivariate spectral calibration methods, two-linear regression-calibration (bivariate calibration (BC)) and multi-linear regression-calibration (MLRC) are proposed for the simultaneous resolution of a binary mixture of hydrochlorothiazide (HCT) and captopril (CTP), which have closely overlapping spectra.

Materials and Methods: This procedure is the mathematical basis of the BC method for two-component analysis. As explained here, this calibration model can be applied easily to resolution of the two-component or binary mixtures. The choice of optimum wavelengths plays an important role in the application of this method to a binary mixture analysis. The aim of the present work is the application of BC and MLRC methods to the resolution of a binary mixture containing hydrochlorothiazide and captopril without requiring a chemical pretreatment and a graphical procedure for the overlapping spectra.

Bivariate calibration method

As an application of this method, 15 wavelengths were considered for the binary mixture systems. The 15 linear regression equations were obtained by using the measurements of the absorbances at 15 wavelengths against the concentrations of standard solution for each compound.

**** Multi-linear regression-calibration method***

This approach is analogous to the BC method, but the MLRC method involves an n-wavelength procedure instead of two-wavelengths.

Results: The absorption spectra of HCT, CTP and their mixture were observed in the spectral region 215–300 nm. Since the spectra of two drugs overlap in the working wavelength range, it is not possible to determine HCT, and CTP simultaneously in their mixture by conventional spectrophotometric methods. In order to solve this problem, the two methods (BC and MLRC) were applied.

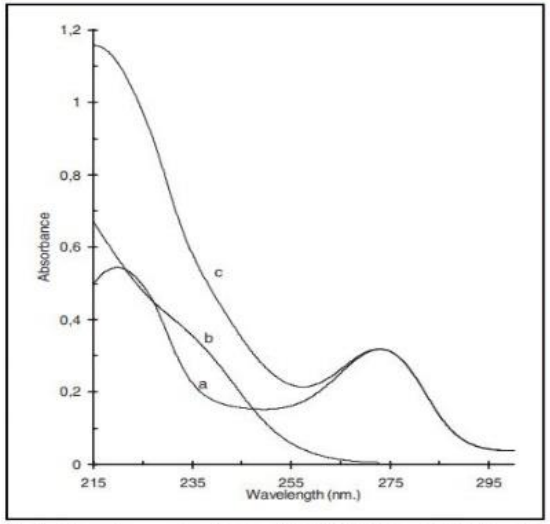

Fig. 1: Absorption spectra of a) 6 µg/mL HCT, b) 16 µg/mL CTP, and c) their mixture in 0.1 M NaOH and methanol (1 : 1).

Table 1: Linear regression analysis and its statistical results at 15 wavelengths

	HCT							CTP						
λ (nm)	Regression equation A = a C + b	(r)	Sr	Sb)	S(a)	LOD µg/mL	LOQ µg/mL	Regression equation	(r)	Sr	Sb)	S(a)	LOD µg/mL	LOQ µg/mL
220	A = 0.0873 C + 0.0119	0.9999	0.15	0.07	0.07	0.021	0.071	A = 0.0375 C − 0.0268	0.9999	0.12	0.05	0.05	0.079	0.265
224	A − 0.0820 C + 0.0153	1.0000	0.15	0.07	0.07	0.028	0.096	A − 0.0329 C − 0.0253	0.9999	0.11	0.04	0.05	0.082	0.273
228	A = 0.0683 C + 0.0145	1.0000	0.14	0.06	0.06	0.030	0.101	A = 0.0294 C − 0.0274	0.9999	0.11	0.04	0.05	0.088	0.295
232	A − 0.0476 C + 0.0091	0.9999	0.12	0.05	0.05	0.015	0.050	A − 0.0291 C − 0.0678	0.9959	0.11	0.04	0.05	0.281	0.937
236	A = 0.0331 C + 0.0101	0.9999	0.10	0.04	0.04	0.021	0.072	A = 0.0235 C − 0.0285	0.9998	0.10	0.04	0.04	0.089	0.295
240	A = 0.0273 C + 0.0079	0.9999	0.09	0.04	0.04	0.016	0.054	A = 0.0194 C − 0.0245	0.9998	0.09	0.03	0.04	0.075	0.251
244	A = 0.0252 C + 0.0056	0.9999	0.09	0.04	0.04	0.010	0.032	A = 0.0145 C − 0.0188	0.9999	0.08	0.03	0.03	0.056	0.186
248	A 0.0241 C + 0.0075	1.0000	0.08	0.04	0.04	0.015	0.051	A 0.0098 C − 0.0119	0.9999	0.06	0.02	0.03	0.035	0.116
252	A − 0.0243 C + 0.0084	0.9998	0.08	0.04	0.04	0.015	0.051	A − 0.0059 C − 0.0060	0.9998	0.05	0.02	0.02	0.020	0.067
256	A − 0.0267 C + 0.0022	0.9998	0.09	0.04	0.04	0.007	0.023	A − 0.0034 C − 0.0020	0.9995	0.04	0.01	0.02	0.008	0.025
260	A − 0.0389 C + 0.0073	1.0000	0.09	0.04	0.04	0.015	0.049	A − 0.0017 C + 0.0006	0.9981	0.03	0.01	0.01	0.004	0.013
264	A = 0.0383 C + 0.0061	0.9999	0.10	0.05	0.05	0.011	0.036	A = 0.0008 C + 0.0019	0.9950	0.02	0.01	0.01	0.006	0.020
268	A = 0.0461 C + 0.0108	1.0000	0.11	0.05	0.05	0.023	0.077	A = 0.0004 C + 0.0013	0.9900	0.01	0.01	0.01	0.004	0.013
272	A = 0.0513 C + 0.0070	1.0000	0.12	0.06	0.05	0.013	0.044	–	–	–	–	–	–	–
276	A − 0.0496 C + 0.0036	0.9999	0.12	0.05	0.05	0.008	0.025	–	–	–	–	–	–	–

C = concentration (µg/mL)
A = Absorbance values at selected wavelength for HCT and CTP

r = Regression coefficient
Sr = Standard deviation of linear regression
Sb) = Standard deviation of slope
S(a) = Standard deviation of intercept
LOD = Limit of detection
LOQ = Limit of quantification

Table 2: Results obtained for the pharmaceutical samples (mg/tablet) by the proposed methods

	HCT (mean ± SD)		CTP (mean ± SD)	
	BC	MLRC	BC	MLRC
Mean	25.4	25.2	51.1	50.7
SD	0.72	0.61	1.47	1.56
RSD	2.83	2.42	2.88	3.08
SE	0.42	0.35	0.85	0.90
CL (p = 0.05)	0.84	0.71	1.71	1.81

SD = Standard deviation, RSD = Relative standard deviation, SE = Standard error, CL = Confidential limit (P = 0.05)

Conclusions: The multivariate spectral calibration methods, two-linear regression-calibration (bivariate calibration (BC)) and multi-linear regression-calibration (MLRC) were applied succesfully for the simultaneous resolution of a binary mixture of hydrochlorothiazide (HCT) and captopril (CTP), which have closely overlapping spectra. The BC and MLRC methods which are very rapid, and easy to apply, yet not expensive, are powerful tools with very simple mathematical contents for the quantitative analysis.

References:

1-Kramer, R (1998). Chemometric techniques in quantitative analysis, Marcel Dekker. Inc. New York pp. 51-61.

2-Beebe, KR., Kowalski, BR. (1987) Anal. Chem. 59, 1007-1017.

3-Adams, MJ. (1995) Chemometrics in analytical spectroscopy, The Royal Society of Chemistry, Thomas, Graham House, Science Park, Cambridge, pp. 187-197.

JUNE 22-25, 2021
ANKARA, TURKEY
ISOPS 13th International SYMPOSIUM ON PHARMACEUTICAL SCIENCES
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APPLICATION OF CHEMOMETRIC TECHNIQUES TO THE CHROMATOGRAPHIC DATA FOR DETERMINATION OF ACTIVE COMPOUNDS IN TABLETS

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Introduction: New multivariate approaches have been applied to high-performance liquid chromatography (HPLC) with multiwavelength photodiode-array (PDA) detection. Multivariate calibration techniques such as classical least squares (CLS), and inverse least squares (ILS) was subjected to HPLC data for simultaneous quantitative analysis of synthetic binary mixtures and a commercial tablet formulation (1–3). Multivariate calibration techniques such as classical least squares (CLS), and inverse least squares (ILS) was subjected to HPLC data for simultaneous quantitative analysis of synthetic binary mixtures and a commercial tablet formulation containing hydrochlorothiazide (HCT) and losartan potassium (LST).

Materials and Methods: Chromatographic separation of the two active compounds, was accomplished by means of a 4.6 mm i.d. × 250 mm, 5 µm particle, Waters Symmetry C18 reversed-phase column and a mobile phase consisting of 60:40 acetate buffer–acetonitrile (v/v, 60:40).

***HPLC–CLS Method**

This method entails application of multilinear regression (MLR) to the peakarea ratios for each analyte. If we consider the responses as peak-area ratios (R) at five wavelengths for six calibration standards for each analyte (concentration set, C).

***HPLC–ILS Method**

This method is the inverse of HPLC–CLS calibration procedure. In this approach, R and C on both sides of equation are replaced for application of multilinear regression to the peak-area ratios for the individual analytes.

Results: The CLS, ILS calibration plots for hydrochlorothiazide and losartan potassium were constructed separately by using the peak-area ratios corresponding to the concentrations of each active compound. These multivariate chromatographic methods were also applied to a commercial pharmaceutical dosage form containing HCT and LST.

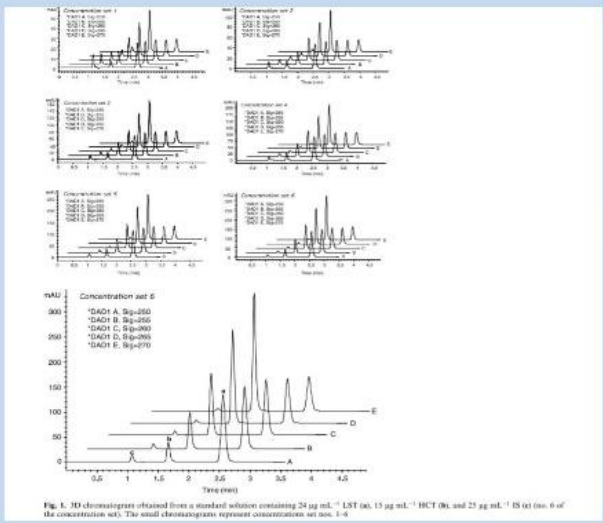

Fig. 1. 3D chromatogram obtained from a standard solution containing 24 µg mL^{-1} LST (a), 15 µg mL^{-1} HCT (b), and 25 µg mL^{-1} IS (c) (no. 6 of the concentration set). The small chromatograms represent concentrations set nos. 1–6

Table 1. Experimental and statistical results obtained by applying the developed methods to commercial tablets

	HPLC–CLS		HPLC–ILS	
	HCT	LST	HCT	LST
Mean	12.50	48.90	12.30	48.50
SD	0.28	0.83	0.18	1.08
RSD	2.24	1.69	1.46	2.23
SE	0.12	0.37	0.08	0.48
CL (P = 0.05)	0.24	0.73	0.16	0.95
ANOVA test	2.31	1.77	2.31	1.77
$F_{theoretical}$	3.00	3.00	3.00	3.00
$F_{calculated}$	0.68	1.27	1.67	0.75
$F_{theoretical}$	6.39	6.39	6.39	6.39
$t_{calculated}$	0.01	0.04	0.25	0.20
$t_{theoretical}$	2.78	2.78	2.78	2.78

n = 5

Label claim: 12.5 mg HCT and 20 mg LST per tablet

Conclusions: For good chromatographic separation and determination it is not always possible to find optimum chromatographic conditions. For this reason, multivariate HPLC calibration has an important role in evaluation of chromatograms acquired at multiple wavelengths by use of a photodiode-array detector. The chemometric calibration methods were applied succesfully for the simultaneous resolution of synthetic binary mixtures and a commercial tablet formulation . The CLS and ILS methods are rapid, easy and powerful tools for the quantitative analysis of hydrochlorothiazide and losartan potassium mixtures and tablets. This new application of multivariate calibration to HPLC data is an alternative means of minimizing experimental errors in chromatographic analysis.

References:

1-Kramer, R (1998). Chemometric techniques in quantitative analysis, Marcel Dekker. Inc. New York pp. 51-61.

2-Beebe, KR., Kowalski, BR. (1987) Anal. Chem. 59, 1007-1017.

3-Haaland DM, Thomas EV (1990) Anal. Chem 62:1091–1099.

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APPLICATION OF MAGNETIC SOLID PHASE EXTRACTION FOR PARABEN RESIDUES IN COSMETIC SAMPLES

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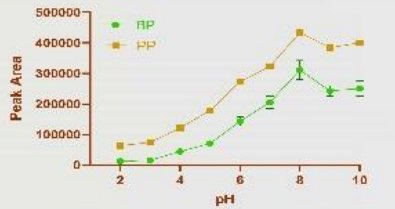
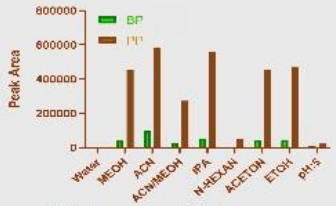
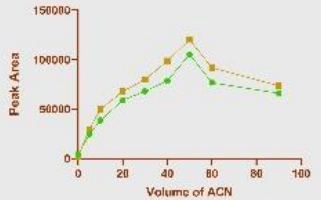
Abstract

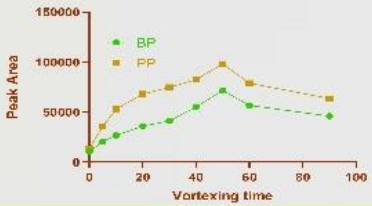
Parabens have been used as preservatives for long time. Currently, they are widely used preservatives, mainly in cosmetics and pharmaceuticals, but also in food commodities and industrial product. The method to be developed is aimed to be based on magnetic solid phase extraction (MSPE), which has been widely used in the literature in recent years and offers application practicality. propyl paraben (PP)benzyl paraben (BzP)were successfully determined. The calibration plots were obtained as linear for both target molecules in the range of with 10-750 ng mL^{-1} with correlation coefficient of 0.9952. Under the optimized conditions, the developed method was applied with satisfactory reproducibility with relative standard deviations less than 4.2 %. Analytical validation of the developed method was carried out by model solutions including PP and BzP molecules at 200 ng mL^{-1}. Finally, application of method was performed by means recovery tests in cosmetic samples.

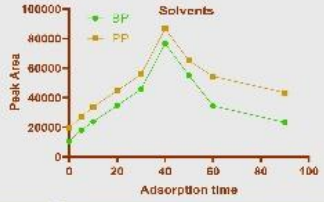
Steps of MSPE

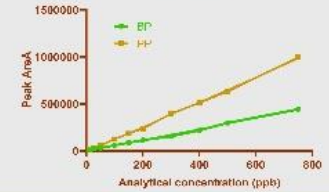












Parameter	Before MSPE		After MSPE	
	BP	PP	BP	PP
Linearity	1.0-70.0 µg mL^{-1}	1.0-70.0 µg mL^{-1}	10-750.0 ng mL^{-1}	10-750.0 ng mL^{-1}
LOD	0.38 µg mL^{-1}	0.42 µg mL^{-1}	3.22 ng mL^{-1}	3.32 ng mL^{-1}
LOQ	0.89 µg mL^{-1}	0.92 µg mL^{-1}	9.52 ng mL^{-1}	9.90 ng mL^{-1}
Rsb (%)	4.2	4.5	3.2	3.1
Calibration Sensitivity	14.5	12.2	1365.2	1125.5
Correlation Coefficient(R)	0.9971	0.9989	0.9984	0.9973
Pre-Concentration Factor	-	-	100	100

Samples	Added ng mL^{-1}	Found ng mL^{-1}		% RSD		% Recovery	
		BP	PP	BP	PP	BP	PP
Facial Cleaning Gel	0.0	<LOD	58.5±2.9	-	4.9	-	-
	100.0	96.7±3.8	104.6±4.5	3.9	4.3	96.7	104.6
	200.0	198.1±9.4	201.5±5.6	4.7	4.3	99.0	102.3
Shampoo	0.0	<LOD	65.8±2.9	-	3.4	-	-
	100.0	96.6±4.4	104.5±5.5	4.5	5.2	96.6	104.5
	200.0	195.5±7.2	203.9±9.1	3.7	4.4	97.7	101.9

DEVELOPMENT AN ANALAYTICAL METHODOLGY FOR ANALYSIS OF NAPROXEN SODIUM AT TRACE LEVELS in BIOLOGICAL SAMPLES BY HPLC-DAD

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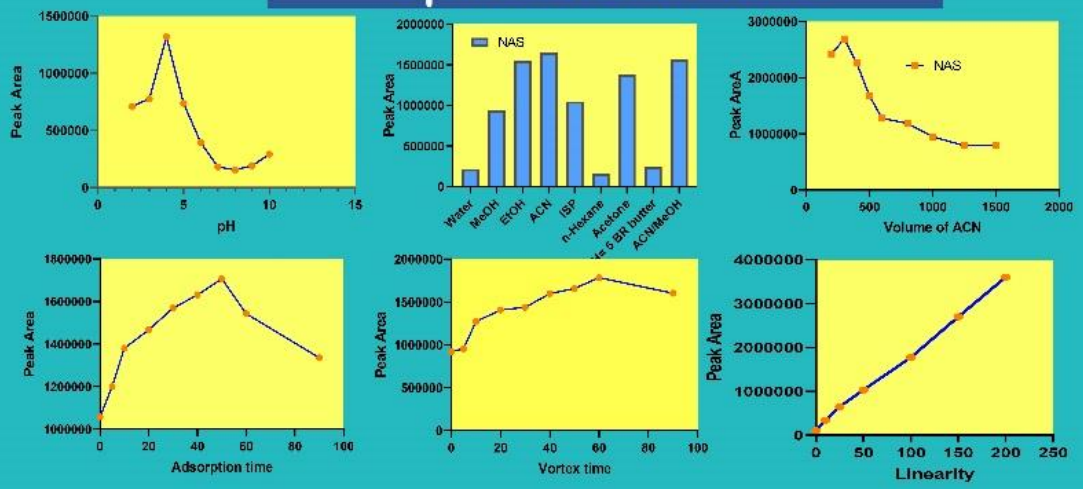
Abstract

Naproxen sodium is a prescription drug used in the treatment of diseases such as pain and inflammation.. A sensitive and easy applicable analytical method was developed for the Naproxen sodium drug residues in simulate urine and normal urine samples using magnetic solid phase extraction (MSPE) followed by HPLC-DAD detector. The quantitative data for Naproxen sodium were obtained via PDA detector at their maximumwavelengths of 219 nm and 256 nm, respectively.This method was developed for the Naproxen reproducibility with relative standard deviations less than 3.5 % and LOD values were lower than 0.35 ng mL-1. Recovery values were calculated by means of spiked samples and found in the range of 95.4-103.8 %. The synhtezied material and developed method have a potential to submit very applicable approaches for determination of NAS residues in urine samples.

Steps of te Proposed Method

Optimization of Data



Analytical Merits of The Developed Method

Parameter	Before MSPE	After MSPE
Linearity	1.0-20.0 μg mL^{-1}	1.0-200.0 ng mL^{-1}
LOD	0.38 μg mL^{-1}	0.35 ng mL^{-1}
LOQ	0.94 μg mL^{-1}	0.95 ng mL^{-1}
RSD (%)	4.4	3.2
Calibration Sensitivity	28.5	4218.5
Correlation Coefficient(R^2)	0.9971	0.9954
Pre-Concentration Factor	-	166

Application of The Developed Method

Samples	Added ng mL^{-1}	Found ng mL^{-1}	% RSD	% Recovery
Simulated Urine	0.0	<LOD	-	-
	50.0	47.7±1.8	3.7	95.4
	100.0	98.1±4.4	4.5	98.1
Normal Urine taken from a Volunteer	0.0	<LOD	-	-
	50.0	52.4±2.0	3.9	104.8
	100.0	105.3±4.8	4.6	105.3

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SENSITIVE DETERMINATION OF KETOPROFEN AND IBUPROFEN in URINE SAMPLES

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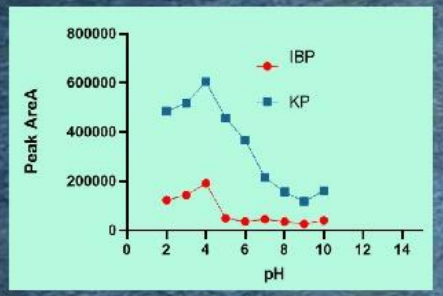
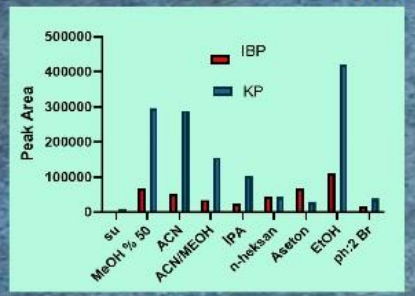
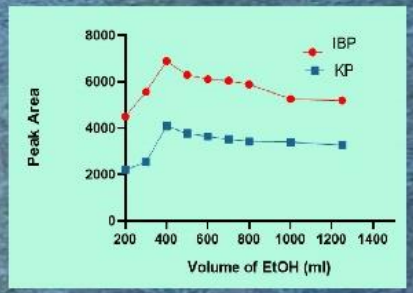
Abstract

Non-steroidal anti-inflammatory drugs can be detected sensitively at very low concentrations in biological and environmental samples. Ibuprofen and ketoprofen are also in this class of drugs. In this study, HPLC-DAD with magnetic solid phase extraction was used to be applied in synthetic and real urine samples. With the photomultiplier tube (PDA) detector used in the study, the maximum wavelengths were measured as 219 nm for ibuprofen and 256 nm for ketoprofen. The calculated correlation coefficients are 0.9886 for IBU and 0.9958 for KET. The relative standard deviation of the method is much lower than 3.5% and the limit of detection (LOD) is 3.48 ng/mL.

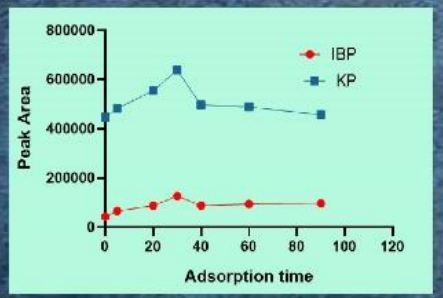
Optimization and Results

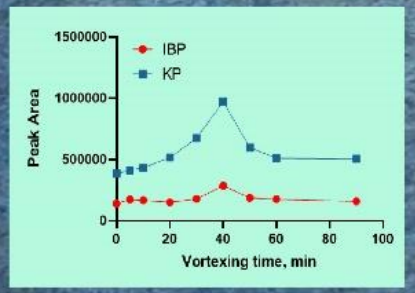


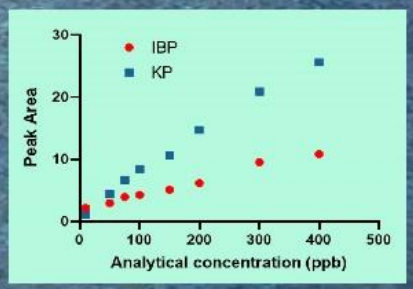




Parameter	Before MSPE		After MSPE	
	KET	IBU	KET	IBU
Linear range	2.0-20.0 μg mL^{-1}	2.0-20.0 μg mL^{-1}	10-500.0 ng mL^{-1}	10-500.0 ng mL^{-1}
LOD	0.65 μg mL^{-1}	0.62 μg mL^{-1}	3.48 ng mL^{-1}	3.43 ng mL^{-1}
LOQ	1.85 μg mL^{-1}	1.92 μg mL^{-1}	9.71 ng mL^{-1}	9.75 ng mL^{-1}
RSD (%)	4.8	4.9	3.2	3.5
Calibration Sensitivity	10,218	1,473	1348,8	173,76
Correlation Coefficient(R^2)	0.9961	0.9980	0.9954	0.9873
Pre-Concentration Factor	-	-	100	100







Samples	Added ng mL^{-1}	Found ng mL^{-1}		% RSD		% Recovery	
		KET	IBU	KET	IBU	KET	IBU
Simulated Urine Samples	0.0	<LOD	<LOD	-	-	-	-
	100.0	98,7±4,1	104,8±4,5	4,2	4,3	98,7	104.8
	250.0	255,1±12,5	240,5±11,5	4,9	4,8	97,0	96.2
Real Urine Samples	0.0	<LOD	<LOD	-	-	-	-
	100.0	99,8±4,8	98,8±3,5	4,8	3,5	99,8	98.8
	250.0	242,5±11,2	255,9±12,8	4,6	5,0	97,0	102.4

Magnetic Solid Phase Extraction

SIMULTANEOUS DETERMINATION OF SOME ANTIFUNGAL PESTICIDES FROM HUMAN BIOLOGICAL SAMPLES BY HPLC

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Introduction: Today, in order to meet the food need arising with the increasing world population, the use of pesticides is widely common in many areas. Fungicide pesticides that used for killing fungi are the most economically important pesticide group. Fungicides are toxic due to their natural chemical structure and are difficult to biodegrade. Pesticides' residue levels on the crops in which fungicides are used can also cause toxic effects on humans. For these reasons, analysis studies to determine the amount of toxic pesticides in human biological samples are important [1]. Therefore, the aim of this study is to demonstrate of a HPLC method for the simultaneous determination of thiram (THR), epoxiconazol (EPO), hexaconazol (HEX), tebuconazol (TEB) and diethofencarb (DIE) pesticides from human serum and urine samples.

Materials and Methods: For HPLC study; an isocratic mobile phase with the flow rate of 1 mL min^{-1} that containing a mixture of methanol: ammonium acetate buffer solution (pH 6.3), 77.5:22.5 (v/v) at 25 °C using LUNA C18 (150 mm × 4.60 mm ID, 5 µm), (Phenomenex, USA) stationary phase with the detection wavelength of 220 nm was used for the separation. A 3600 µL aliquot of human blank serum and urine samples were mixed with sufficient amount from THR, EPO, HEX, TEB and DIE stock solutions. The final volume was completed to 10 mL acetonitrile and was vortexed for 3 min. The sample was centrifuged for 15 min at 5000 rpm. Hundred microlitre of supernatant solution was then transferred to a glass HPLC vial for analysis.

Results and Discussion:

Mobile Phase Composition

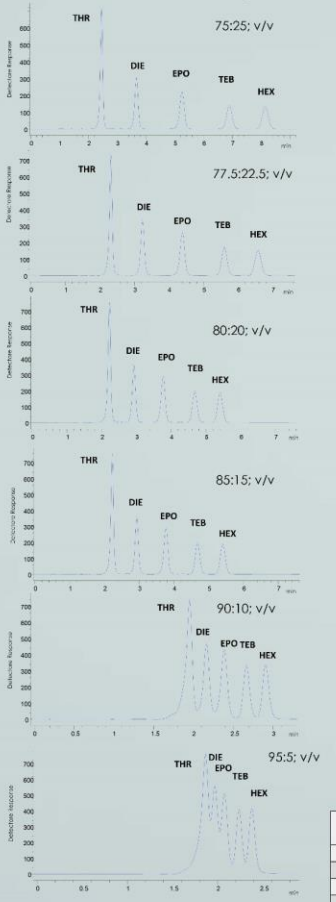

Fig.1 Luna C-18 (150 mm x 4.6 mm , 5 µm), 25 °C, 1mL min^{-1} flow rate, 220 nm

Flow Rate Optimization

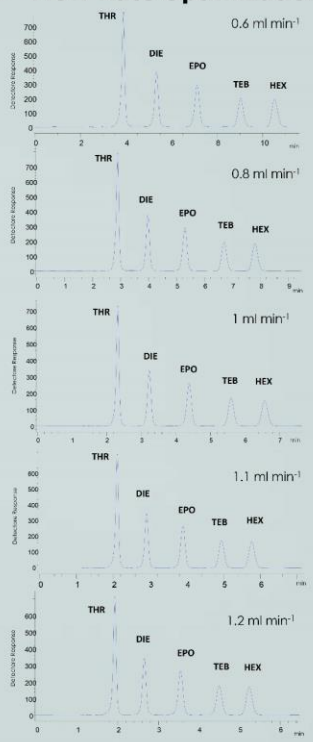

Fig.2 Luna C-18 (150 mm x 4.6 mm , 5 µm), pH:6.3, 25 °C, 220 nm, MET:Buffer (77.5:22.5; v/v) compositions.

Temparature Optimization

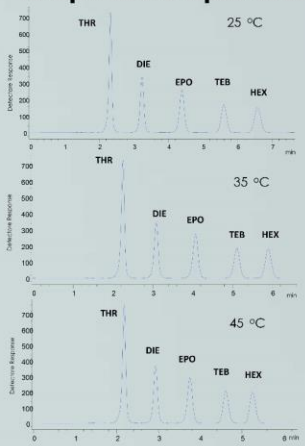

Fig. 3 Luna C-18 (150 mm x 4.6 mm , 5 µm), pH:6.3, 1mL min^{-1} flow rate 220 nm, MET:Buffer (77.5:22.5; v/v) compositions.

Calibration Results

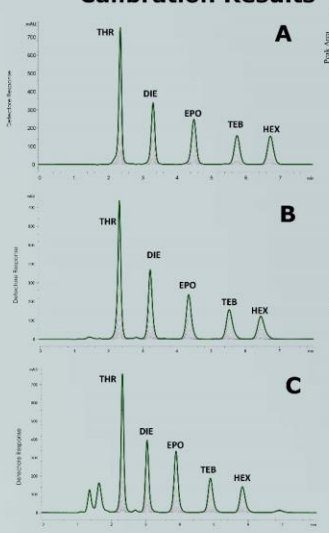

Fig. 4 Calibrations results by using optimized conditions for solvent (A), serum (B) and urine (C)

Calibration Curves

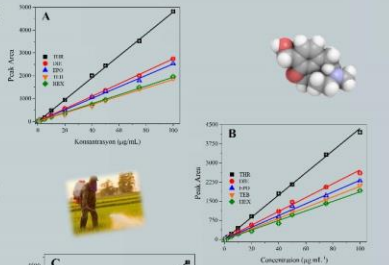

Fig. 5 Calibrations curves by using optimized conditions for solvent (A), serum (B) and urine (C)

Table 1. Validations parameters in solvent found as a result of calibration calculations

Validation parameters	Solvent				
	THR	DIE	EPO	TEB	HEX
Linearity range (µg mL^{-1})	0,5-100				
Slope	47,98	27,39	25,13	18,30	19,27
Intercept	-11,54	2,20	3,89	1,43	7,24
Correlation coefficient	0,997	0,999	0,997	0,995	0,998
SE of slope	1,08	0,21	0,38	0,42	0,25
SE of intercept	4,05	1,00	3,00	8,01	2,31
Limit of Detection (µg mL^{-1})	0,04	0,07	0,08	0,07	0,09
Limit of Quantification (µg mL^{-1})	0,10	0,20	0,24	0,20	0,26
Within-day precision[a] (RSD %)*	0,21	0,47	0,98	1,11	1,15
Between-day precision[a] (RSD %)*	0,40	0,68	1,12	1,20	1,35

Table 2. Validations parameters in serum samples found as a result of calibration calculations

Validation parameters	Serum				
	THR	DIE	EPO	TEB	HEX
Linearity range (µg mL^{-1})	0,5-100				
Slope	43,48	27,03	23,04	20,81	18,66
Intercept	-0,64	17,24	14,61	-1,43	-2,35
Correlation coefficient	0,998	0,997	0,993	0,998	0,994
SE of slope	0,42	0,44	0,64	0,30	0,47
SE of intercept	2,51	2,70	4,40	1,99	2,29
Limit of Detection (µg mL^{-1})	0,03	0,07	0,12	0,12	0,08
Limit of Quantification (µg mL^{-1})	0,07	0,21	0,36	0,35	0,24
Within-day precision[a] (RSD %)*	0,33	0,99	1,28	1,68	1,99
Between-day precision[a] (RSD %)*	0,43	1,24	1,97	1,99	2,15

Table 3. Validations parameters in urine samples found as a result of calibration calculations

Validation parameters	Urine				
	THR	DIE	EPO	TEB	HEX
Linearity range (µg mL^{-1})	0,5-100				
Slope	46,71	29,68	27,69	19,55	16,97
Intercept	-13,86	-4,35	-0,50	1,31	7,24
Correlation coefficient	0,998	0,997	0,999	0,999	0,994
SE of slope	0,64	0,48	0,35	0,18	0,44
SE of intercept	2,27	5,40	2,87	1,60	2,19
Limit of Detection (µg mL^{-1})	0,01	0,08	0,07	0,12	0,12
Limit of Quantification (µg mL^{-1})	0,04	0,25	0,22	0,36	0,37
Within-day precision[a] (RSD %)*	0,34	0,91	1,27	1,73	1,99
Between-day precision[a] (RSD %)*	0,53	1,37	1,43	2,18	2,38

Conclusions: The linearity of the proposed method in human serum and urine samples were determined by analysis of different concentrations levels of each pesticides (Fig. 1). The linear ranges were found 0.5–100.0 µg mL^{-1} in all matrix. To demonstrate of method sensitivity, limit of detection (LOD) values were calculated from the equations where the standard deviation of response and the slope of the calibration curve were used. LOD values were found to be between 0.03 and 0.08 µg mL^{-1} for serum samples, 0.01 and 0.12 µg mL^{-1} for urine samples. Moreover, when intra-day and inter-day RSD values (less than 4%) were examined, it was seen that repeatability results were obtained in all matrix. In this work, HPLC method was applied for the simultaneous separation and determination of some antifungal pesticides from human serum and urine samples. The developed method was rapid, highly feasible, reproducible and can be used in research and development laboratories as well as the quality control laboratories where the high throughput analysis is required.

Acknowledgements
This work was produced as a part of B. Bugra Barut's PhD thesis. The authors would like to thank Ankara University Scientific Research Projects Commission for financial support (Project No: 20L0237007)

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JUNE 22-25, 2021
ANKARA, TURKEY
ISOPS 13th International SYMPOSIUM ON PHARMACEUTICAL SCIENCES