RESEARCH ARTICLE

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Liquid Chromatographic-Mass Spectrometric Analysis of Diclofenac Based Cu (II) Complex

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ABSTRACT: Coordination compounds have an important place in the structure of living things. Because; coordination compounds have an important place in pharmacology, biological reactions such as electron transfers and reaction mechanisms of enzymes as co-factors of many enzymes. Copper, which has many functions in the human body, also has many benefits. Copper, which plays a role in the functioning of the central nervous system, is also effective in the formation of free energy in the body. Copper is also found in many enzymes and plays a role in protein synthesis in the body. Anemia, fatigue and weakness, weakness of the immune system, iron deficiency, loss of appetite and similar disorders are seen in iron deficiency. In our study, which has very few examples in the literature, bis {2- [2-(2,6-dichloroanilino) phenyl] acetato-κ^2 O, O'} bis (1-vinly-1H-imidazole- κN^3) copper (II) complex has been studied. In our study, it was investigated whether the axial ligands are replaced by the solvent when the synthesized diclofenac-based copper complex is dissolved in different solvents. When the diclofenac-based copper complex were dissolved in different solvents such as methanol, ethanol and acetonitrile, UV-VIS and FT-IR measurements were taken and their behaviour was examined. Mass confirmations of the new compounds formed by measurements using liquid chromatography mass spectrometry device were made. When the measurement results obtained using different measurement techniques are compared, it is seen that the results support each other.

Keywords: Coordination compounds, Diclofenac based Cu (II) complex, Liquid chromatographic-mass spectrometric analysis

INTRODUCTION

Coordination compounds are formed when a central atom is surrounded by a series of neutral or negatively charged atoms or groups, called ligands that have an electron excess. There are some important coordination compounds in the structure of living things. The most important biological complex of Fe (II) ions is haemoglobin. The chlorophyll substance found in the structure of plants and giving them a green color is the coordination compound whose central atom is magnesium. Coordination compounds have an important place in pharmacology. Some drugs have coordinated bonds in their mechanism of action.

One of the metals widely used in coordination compounds is copper. Copper is among the most abundant transition metals in the human body and plays various roles in human physiology. While copper is at the active center of enzymes responsible for numerous redox processes, its excess or deficiency can cause diseases such as Wilson's disease or Menkes syndrome (Crisponi et al.,2010; Chellan et al., 2015).

Copper plays a role in the work of the central nervous system, in the formation of free energy in the body, in the structure of many enzymes and in protein synthesis. In case of copper deficiency in our body; anemia, fatigue, weakness in the immune system, iron deficiency, loss of appetite and similar disorders are seen.

Electrospray ionization-mass spectrometry (ESI-MS) is one of the popular and powerful methods used to identify and determine organic and inorganic ions, especially preferred as a detector for ion chromatography and HPLC. The most characteristic feature of this method is that it is a soft ionization technique. Therefore, metal-organic complexes can also be detected (Fenn et al., 1989; Smith et al., 1990; Traeger, 2000; Umemura et al., 2001; Hotta et al., 2008; Hotta et al., 2009; Keith-Roach, 2010).

Electrospray ionization mass spectrometry (ESI-MS) (Yamashita et al., 1984; Fenn et al., 1990; Cole, 1997; Cole, 2000) is an effective tool for the analysis of a large number of non-covalent complexes such as host-guest chemistry today (Loo, 2000; Schalley,2000; Shen et al., 2000). ESI-MS analysis allows the determination of complexes formed in solution and examination of their equilibrium states, quantitative analysis of stability constants or evaluation of their binding selectivity (Bartsch and Eley, 1996; Krabbe et al., 1996; Scherman et al., 2003).

The high selectivity and simultaneous analysis of multiple species is one of the most striking features of this method. Transition metals as copper, iron and zinc, play a vital role in the metabolism of living organisms. ESI-MS is used because of the identification of metal complexes in a wide variety of samples, its high selectivity, sensitivity and smooth transition from solution to gas phase (Meija et al., 2006; Keith-Roach, 2010).

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MATERIALS AND METHOD

Reagents

Methanol were purchased from Merck, ethanol and acetonitrile were purchased from J.T.Baker and their purity was LC/MS grade. All the chemicals used in this study were in the analytical grade and were used without further purification. The solutions required for the measurements were prepared by dissolving the complexes in methanol, ethanol and acetonitrile.

Sample Solution

The diclofenac-based copper complex we used in our study, [Cu $(dicl)_2(vim)_2$], was synthesized by Assoc. Dr. Sevim Hamamcı Alisir (Alisir et al.,2019). FT-IR and UV spectra of the synthesized complex were obtained in methanol, ethanol and acetonitrile solvents. Mass confirmations were made using liquid chromatography-mass spectrometry. In Figure 1, the shape and formula of the complex we used in our study are given.

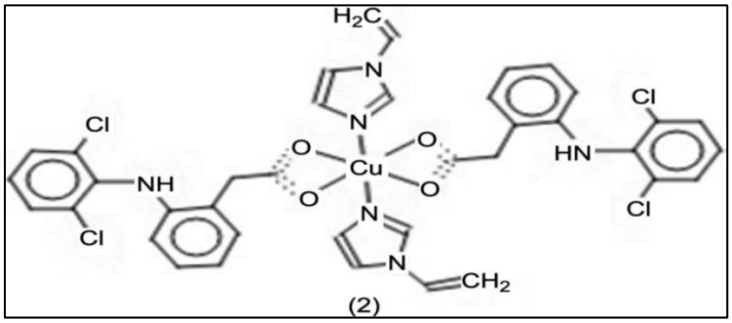

Figure1. *Bis{2-[2-(2,6-dichloroanilino)phenyl]acetato-κ^2O,O'}bis(1-vinly-1H-imidazole-κN^3) copper(II), $[Cu(C_{14}H_{10}Cl_2NO_2)_2(C_5H_6N_2)_2]$, $[Cu(dicl)_2(vim)_2]$*

Samples were prepared during analysis, as the replacement of ligands in the complex with solvent molecules was very rapid and the complex was likely to deteriorate during prolonged standing. For LC-MS/MS analyzes, solutions prepared in methanol, ethanol and acetonitrile were injected into the system by filtering through syringe tip filter (0.45 µM nylon). LC-MS/MS analyzes were studied in both positive and negative modes.

Chromatographic conditions

The study was carried out using the Thermo Scientific brand TSQ Quantum Access Max model LC-MS/MS device. For liquid chromatography-tandem mass spectrometer analysis, LC-MS/MS device was used at Hitit University Scientific Technical Application and Research Center (HÜBTUAM) and analyzes were performed at HÜBTUAM. For the mobile phase, acetonitrile, methanol and ethanol were used separately. The mobile phase was filtered through a 0.45 µm pore size membrane filter and sonicated 15 min before use. The flow rate of the mobile phase was held at 0.2 mL min^{-1} and the injection volume was set as 20 µL. Analysis time was 3 minute. Sample information and chromatographic conditions are given in Table 1.

Table 1.*Sample information*

Name of the product	Synthesis products	
Product material	Liquid	
	Minute	% A (Methanol-ethanol-acetonitrile)
Solvent program	0	100
	3	100
Solvent flow rate	0,2 mL/min	
Column oven temperature	25 ºC	
Column features	-	
Injection volume	20 µL	
Analysis time	3 min	
Standards	Synthesis products	

MS/MS analysis conditions

The mass analyzer operated with an ESI source positive/negative ion mode, Cappilary Temperature: 300 ºC, Vaporizer Temperature: 350 ºC, Sheat Gas Pressure (Arb): 25, Aux Gas Pressure (Arb): 10, Sprey Voltage (V) Pozitive Polarity: 4400, Sprey Voltage (V) Negative Polarity: 2500, Discharge Current (µA): 4,0 in positive/negative polarity. MS/MS device conditions are given in Table 2.

Table 2. *Analysis test conditions*

MS/MS analysis conditions	
Capillary temperature	300 ºC
Vaporizer temperature	350 ºC
Sheeth gas pressure (Arb)	25
Aux gas pressure (Arb)	10
Spray voltage (positive polarity)	4400
Spray voltage (negative polarity)	2500
Discharge current (positive polarity)	4
Discharge current (negative polarity)	4
Full scan mode	100-1500 m/z
Ion mode	negative/positive polarity
Ionization source	ESI

UV measurements of the complexes were made with Thermo Scientific Evolution Array UV-VIS Spectrophotometer. FTIR measurements of the complexes were made using the Perkin Elmer Spectrum Two device.

RESULTS

In our study, it was investigated whether the axial ligands were replaced by solvent molecules when the synthesized diclofenac-based copper II complex was dissolved in different solvents. Therefore, the behavior of the synthesized complex in methanol, ethanol and acetonitrile was investigated. The mass spectrum of the complex synthesized in the study was taken in both positive and negative ESI mode in different solvents. The mass spectrum obtained using different solvents was compared. In Figures 2, 3 and 4 the mass spectra obtained in methanol, ethanol and acetonitrile are given.

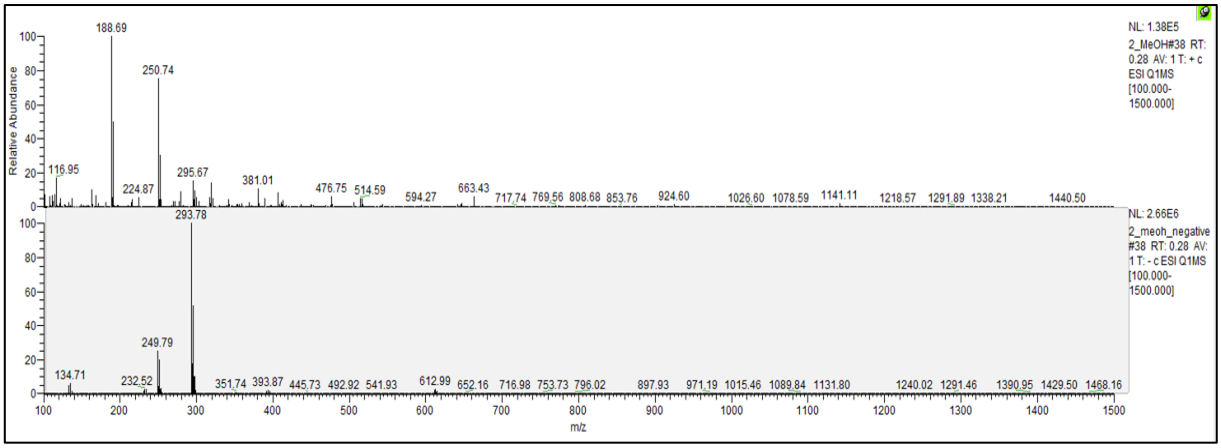

Figure 2. *Mass spectrum of the complex of [Cu(dicl)$_2$(vim)$_2$] in positive and negative ESI mode in methanol*

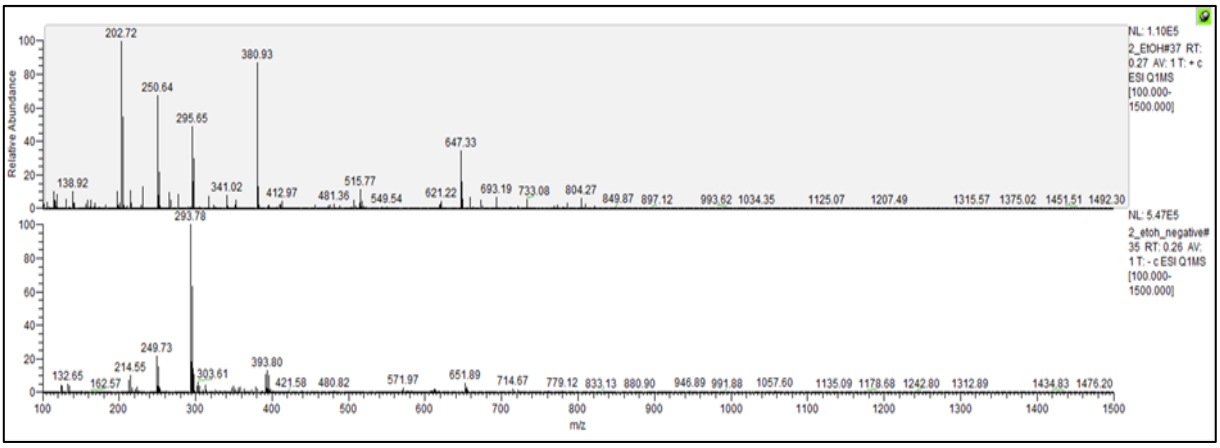

Figure 3. *Mass spectrum of the complex of [Cu(dicl)$_2$(vim)$_2$] in positive and negative ESI mode in ethanol*

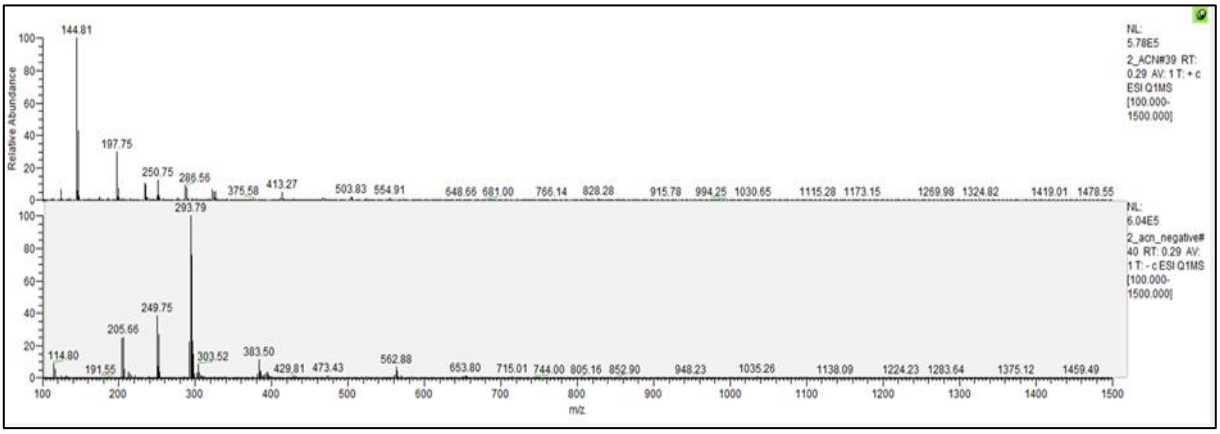

Figure 4. *Mass spectrum of the [Cu(dicl)$_2$(vim)$_2$] complex in acetonitrile in positive and negative ESI mode.*

When the mass spectrum obtained in positive and negative ESI mode of the synthesized [Cu(dicl)$_2$(vim)$_2$] complex in methanol was examined, it was seen that the complex did not replace the solvent in both ESI modes. When the mass spectrum of the [Cu(dicl)$_2$(vim)$_2$] complex was analyzed when ethanol was used as solvent, it was seen that its three ligands were replaced by ethanol in negative ESI mode. When the mass spectrum obtained in negative ESI mode was examined, it was seen that the three ligands changed with the solvent in the negative ESI mode. It was observed that the complex did not displace methanol in both ESI modes, whereas ethanol and acetonitrile only replaced the solvent in negative ESI mode.

The UV spectra for the [Cu(dicl)$_2$(vim)$_2$] complex in methanol, ethanol and acetonitrile are given in Figures 5, 6 and 7. When the UV spectra of [Cu(dicl)$_2$(vim)$_2$] complex obtained in different solvents are examined; peaks are seen at 647.20 nm and 665.20 nm. In copper metal, there were shifts in d-d transitions due to differences in polarity of solvents.

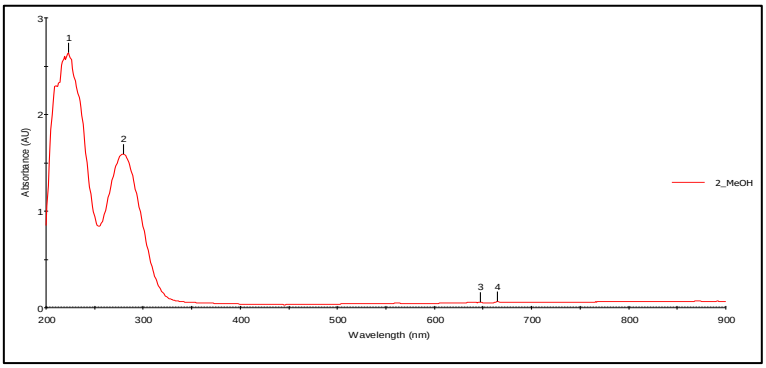

Figure 5. *UV spectrum obtained in methanol for [Cu (dicl)$_2$(vim)$_2$] complex*

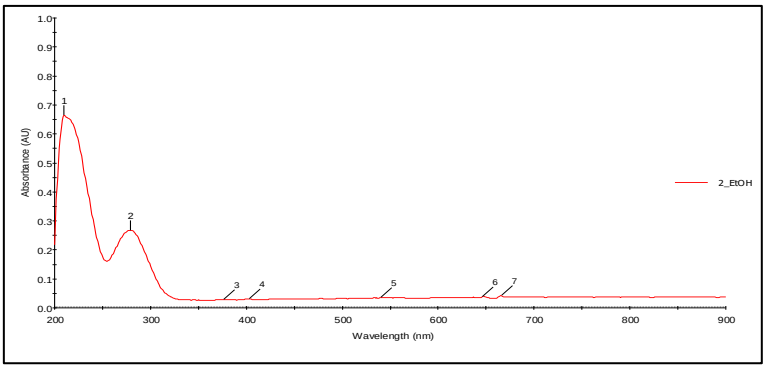

Figure 6. *UV spectrum obtained in ethanol for [Cu(dicl)$_2$(vim)$_2$] complex*

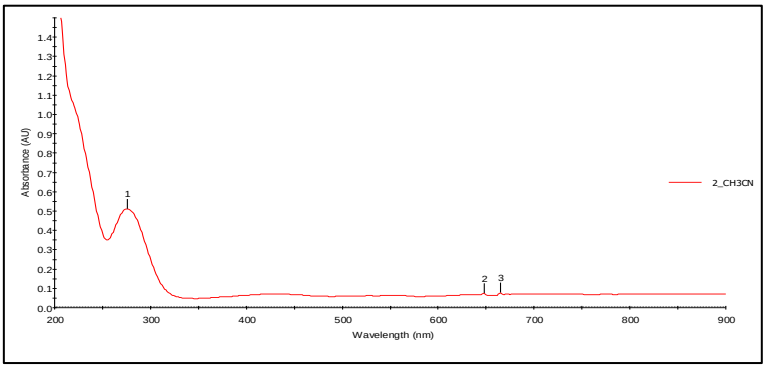

Figure 7. *UV spectrum obtained in acetonitrile for [Cu(dicl)$_2$(vim)$_2$] complex*

The IR spectrum for the [Cu(dicl)$_2$(vim)$_2$] complex is given in Fgures 8. When the IR spectrum was examined, medium intensity C-O peak at 1231 cm^{-1}, aromatic C= C peak at 1574 cm^{-1}, aromatic C-H peak at 3140 cm^{-1}, medium intensity C-N peak at 1103 cm^{-1}. When the IR spectrum of the complex was examined, it was seen that the obtained values were compatible with the literature.

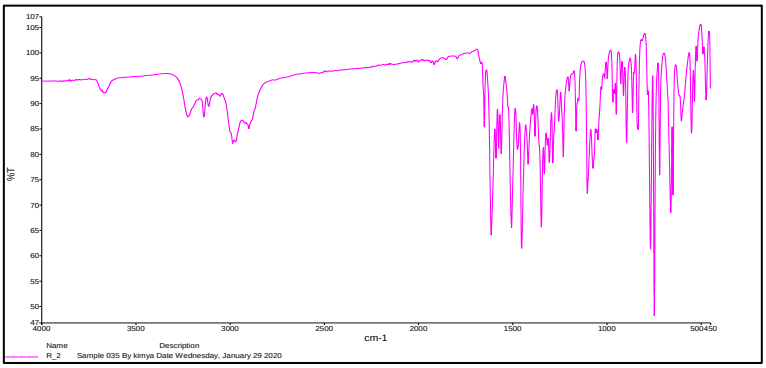

Figure 8. *IR spectrum of [Cu(dicl)$_2$(vim)$_2$] complex*

DISCUSSION

The behaviour of axial ligands of the synthesized diclofenac-based copper II complex in different solvents was investigated. Analysis time was short (3 minutes) and the behaviour of the ligands was examined by mass verification. Considering that the behaviour of axial ligands against different solvents in the literature is not investigated using the LC-MS / MS device, the specificity of the study is revealed. In other words, they completely transform into different species in which the solvent molecules act as ligands.

CONFLICT OF INTEREST

No conflict of interest.

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