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SPECTROPHOTOMETRIC DETERMINATION OF DILTIAZEM IN PHARMACEUTICAL AND *IN-VIVO* SAMPLES WITH Pd(II)

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ABSTRACT: An analytical method has been developed for quantitative determination of diltiazem, chemically (+)-cis-1,5-benzothiazepin-4-(5H)one, 3-(acetyloxy)-5-[2-(dimethyl amino)ethyl]-2,3-dihydro-2-(4-methoxyphenyl)-monohydro chloride, by complexation with Pd(II) spectrophotometrically. Diltiazem is a calcium channel blocker type antihypertensive drug. Diltiazem forms an stable orange colored 1:2 complex with Palladium(II) Chloride with λmax 400nm, molar absorptivity coefficient of complex ε= 8.5 X10^2 l $mol^{-1}cm^{-1}$, Beer's law range 3.413 X10^1 µg/ml to 2.722 X10^2 µg/ml with intercept of regression 0.019 and correlation coefficient 0.989. Interference of foreign metal ions and effect of temperature and pH was also studied. The characterization of complex involves, Elemental Analysis, FTIR, ^{1}H NMR, ESR, Raman spectra, magnetic susceptibility measurement and thermal studies. On the basis of above studies structure of the complex has been proposed. The developed analytical method was applied on *in-vivo* blood samples- whole blood and serum samples. For whole blood samples Beer's law range is 1.01 X 10^2 µg/ml to 2.77 X 10^2 µg/ml with coefficient of variance ±1.49 and relative standard deviation 0.64% and for serum samples Beer's law range is 1.79X10^2 µg/ml to 2.47 X 10^2 µg/ml with coefficient of variance ±1.03 and relative standard deviation 0.79%. The procedure is rapid, accurate with precision and can be used by pathologists and in industrial sectors for determination and quality test of diltiazem in pharmaceutical samples.

INTRODUCTION: Diltiazem is an important coronary vasodilator drug of the calcium channel blocker type. Chemically, diltiazem HCl is, (+)-cis-1,5-benzothiazepin-4-(5H)one,3-(acetyloxy)-5-[2-(dimethylamino)ethyl]-2,3-dihydro-2-(4-methoxy phenyl)-monohydrochloride [1].

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Chiral resolution of optical isomers of diltiazem molecule have already been achieved using capillary zone electrophoresis [2], electro kinetic chromatography [3] and by HPLC [4-5]. Biological activity of diltiazem as antihypertensive agent and against acute myocardial infection and cardiac arrest [6] has been studied.

Quantitative determination of diltiazem using chromatographic techniques [7-9], stripping voltammetry, FT-Raman spectroscopy [10] and by spectrophotometric methods [11-13] have been reported.

But, the methods reported need sophisticated instrumentation and also many parameters like temperature, pH, flow rate, potential applied etc have to be calibrated to get the accurate and reproducible results.

The present paper reports a spectrophotometric method for the determination of diltiazem using Pd(II) chloride as reagent.

EXPERIMENTAL:

Apparatus: The absorbance was measured on Helicos delta spectrophotometer with one cm glass cells. Elemental analysis was carried out on Elemental Analyzer Carbo Erba 1108. The ESR spectra were recorded in Varian ESR spectrometer in the scan range of 3000 gauss using tetra cynoethyline marker.

FTIR spectra were recorded in Nicolet FTIR spectrophotometer in the range 4000-400cm^{-1} using KBr pellets. ^1H NMR spectra were recorded in varian-300MHz spectrometer using deuterium oxide as solvent. Laser Raman spectra were recorded in -Ramanor HG 25 using Argon Laser (488nm) as a source of irradiation. Magnetic Susceptibility study of the complex was carried using vibrating sample magnetometer EG and G Model: 155. Thermo gravimetric Analysis (TGA) was carried out using Shimadzu TGA-50H having temperature range upto1500^oC with heating rate 0.10^oC/hour.

REAGENTS AND SAMPLES:

Diltiazem Solution – For preparation of standard solution of Diltiazem accurately weighed 120mg of drug and dissolve in 100ml of double distilled water. Standardize the solution Spectrophoto-metrically using hydroxylamine reagent.

Palladium Chloride Solution: To prepare standard solution of Palladium Chloride dissolve 1.0gm of $PdCl_2$ in 1000ml of double distilled water containing 0.25M hydrochloric acid. Dilute the solution to contain required concentration of metal.

Whole Blood Sample: Two blood samples are to be collected from patient suffering from hypertension. First blood sample, 10.0ml without drug, is to be collected before giving the drug. Second blood sample, 10ml with drug, is to be collected after 30mins of administration of diltiazem 60mg tablet. All samples are frozen in dry ice bath until analyzed.

Serum Sample: Serum sample are separated by treating pure blood and drug containing blood separately with 1.0ml of 2% trichloroacetic acid. Sample is then centrifuged for 45 mins at 360 rpm. The supernatant clean liquid is serum.

Optimization of the conditions: Diltiazem solution when mixed with Pd(II) solution at 40^oC forms dark orange colored complex within 90mins having λ_{max} 400nm.Maximum and constant absorbance was obtained in the pH range 6.0 to 7.5 and temperature range 30^oC to 45^oC. All the experimental work was carried out at pH 7.0 and at temperature 40^oC.

COMPOSITION OF COMPLEX: Composition of the complex was determined by Job's method of continuous variation and Mole Ratio method. Metal: Ligand ratio for Palladium(II) chloride : Diltiazem complex was found to be 1:2. The method involves stepwise complexation with stoichiometry as given in the following equation;

$$2(C_{22}H_{38}N_2O_4S.HCl) + PdCl_2 + \longrightarrow xH_2O$$
$$[(C_{22}H_{38}N_2O_4S)_2(H_2O)_2Pd]^{2+}.2Cl^-.2H_2O$$

Procedure for determination:

Analysis of Diltiazem in pharmaceutical samples: To different aliquots from 0.8 ml to 5.0 ml , containing 3.4129 X10^1μg to 1.081 X10^2 μg of drug diltiazem, excess amount of palladium chloride solution having molarity 4.0342 X10^{-4} M was added. The volume was made upto 10ml with double distilled water. The absorbances of different sets were recorded at λmax of complex (400nm), after allowing the solutions to stand for 90 mins. A Beer's Lambert's Graph was plotted by taking known concentration of diltiazem on X-axis and absorbance on Y-axis. The unknown concentration of the complex formed with diltiazem is calculated from the plot.

Analysis of Diltiazem in drug injected whole blood and serum samples: The aliquots 0.6- 2.0 ml whole blood without drug were taken in 50ml Erlenmeyer flasks.

A constant volume (1 ml) of 3.217 X 10^{-3} M palladium chloride was added to all the aliquots. Each aliquot was then diluted to 10ml with double distilled water. Identical sets with variable volume of whole blood sample were arranged and absorbance of each set was recorded at 400nm, the λ max of Pd(II): diltiazem complex. Similar sets were arranged for the whole blood samples with drug. Difference in absorbance values were compared from both the graph and the amount of drug assimilated in whole blood samples were quantitatively determined using the Beer-Lambert's law graph already plotted with known concentration of drug. Same procedure is followed for serum without drug and serum with drug samples.

Characterization of complex:

Elemental analysis results are in good agreement with calculated values. Percentage of C, H, N and S confirm the M: L ratio to be 1:2. Results are summarized in table 1 below.

TABLE 1: RESULT OF ELEMENTAL STUDY OF Pd(II): DILTIAZEM COMPLEX

Element	Calculated	Found
Carbon %	51.2%	51.0%
Nitrogen %	5.4 %	5.7 %
Hydrogen %	8.2%	7.9%
Sulphur %	6.2%	6.0%

Fourier Transformation Infrared (FTIR) spectra of diltiazem and its Pd(II) complex as KBr pellets was recorded in the range 400-4000cm^{-1}. Bond formation between Pd(II) and carbonyl group of Diltiazem during complex formation is confirmed by appearance of new peaks at 1036.5cm^{-1} and 904.1cm^{-1} and by shifting of C=O stretching band from 1656.59cm^{-1} to 1667.9cm^{-1}. –N-C=O stretching band at 2369.9cm^{-1} gets disappeared and stretching band of –C=N gets shifted to higher frequency. Bands at 3305.4cm^{-1} and 634.2cm^{-1} are assigned to bending and stretching modes of H_2O molecule.

Proton Nuclear Magnetic Resonance (^{1}H NMR) spectra of pure diltiazem and Pd(II):Diltiazem complex were recorded at 299.9MHz in D_2O. Bonding of C=O group with Pd(II) is confirmed by shifting of CH-C=O peak from 2.87ppm to 3.14ppm and shifting of –CO-N-CH_2 peak from 3.7 ppm to 3.8ppm. Signals at 4.57, 4.53, 4.36 and 4.34 ppm confirms the presence of water molecules in the complex.

Laser Raman spectral study of the samples was carried out in the range 50-4000cm^{-1}. Shifting of C=O stretching peak from 1740 cm^{-1} to 1643 cm^{-1} with reduced intensity confirm the involvement of C=O in bonding with Pd(II). Presence of coordinated water molecule in the complex is shown by bands at 581.2 cm^{-1} and 3801.6 cm^{-1} assigned to asymmetric and symmetric stretching of –OH group.

Electron Spin Resonance (ESR) spectra were recorded in the scan range 3000gauss using tetra cynoethylene as marker. Diltiazem being diamagnetic in nature do not give ESR signal. ESR spectrum of Pd(II): Diltiazem complex shows hyperfine splitting with total seven hyperfine signals. Value of 'g' for the complex is 1.91 which is quite low as compared to the 'g' value of free electron which indicates covalent nature of the complex.

Magnetic Susceptibility Measurement of the complex was carried out using VSM. The value of μ_{eff} for Pd(II) diltiazem complex as calculated from magnetic field-magnetic moment graph, has been found to be 2.488 BM. This shows that hybridization state of central metal ion Pd^{2+}, (having valency shell electronic configuration $4d^8$ (t_{2g}^6 eg^2)) is sp^3d^2. Thus Pd(II): diltiazem complex is an outer orbital complex having octahedral geometry.

Thermo Gravimetric Analysis (TGA) of Pd(II):Diltiazem complex was carried out in nitrogen atmosphere at a rate of 20^{o}C/min from 0^0C to 600^0C Loss of 17% weight near 100^{o}C corresponds to removal of lattice water molecule. Two weight loss of 16% and 18% at 203^{o}C and 264^{o}C respectively corresponds to loss of coordinated water molecules from the complex. The breakage of drug moiety from the complex starts from 442^{o}C and gets completed at 520^{o}C, with 42% weight loss leaving behind palladium metal and elemental carbon as residue.

RESULTS AND DISCUSSION: A calibration curve for diltiazem was constructed by the standard procedure. A good linear relationship was observed

over the range 3.413 X 10^1 µg/ml to 2.722 X10^3 µg/ml of diltiazem with effective molar absorptivity (ε) 8.5 X 10^2 l/mol cm and relative standard deviation (RSD%) ±0.892%. Regression equation for the Pd(II): diltiazem complex is, Y = 0.0191 + 0.1152 X, and correlation coefficient (r) was found to be 0.989. The analytical method developed for quantitative determination of Diltiazem by forming stable 1:2 complex with Pd(II) was applied on *in-vivo* blood samples. Determination of the drug in whole blood samples using palladium chloride as reagent was linear in the range 1.01 X 10^2 µg/ml to 2.77 X 10^2 µg/ml with coefficient of variance ±1.49 and relative standard deviation is 0.64%. Determination of the drug in serum samples using developed method follow Beer's law in the range1.79X10^2 µg/ml to 2.47 X 10^2 µg/ml with coefficient of variance ±1.03 and relative standard deviation is 0.79%. Interference caused by various diverse ions on the determination of Diltiazem was examined and results are summarized in **table 2**.

TABLE 2: EFFECT OF DIVERSE IONS ON COMPLEXATION

S. No.	Ion	Added as	Tolerance Limit in 1ppm of Diltiazem	% Recovery
1	Cu (II)	$CuSO_4$	16.17 ppm	99.7%
2	Co (II)	$CoSO_4$	12.14 ppm	99.9%
3	Ni(II)	$NiSO_4$	66.70ppm	96.6%
4	Fe(II)	$FeSO_4$	8.40 ppm	97.9%
5	Fe(III)	$(NH_4)FeSO_4$	6.25 ppm	96.5%
6	Mg(II)	MgO	17.42ppm	99.6%
7	Cr(IV)	CrO_3	0.02ppm	93.4%
8	Pb(II)	$Pb(NO_3)$	14.32 ppm	99.5%
9	Cd(II)	$CdCO_3$	0.37 ppm	99.5%
10	Zn(II)	$ZnSO_4$	87.63 ppm	99.1 %

Fourier transformation infra-red (FTIR) spectral studies and proton nuclear magnetic resonance (1H NMR) spectroscopic technique was adopted for further quantitative measurement of *in-vivo* preparation of complex. Results are summarized in **tables 3, 4 and 5**.

TABLE 3: CHARACTERSTIC FTIR BANDS OF PURE WHOLE BLOOD, DRUG INJECTED WHOLE BLOOD AND DRUG INJECTED WHOLE BLOOD WITH Pd(II)

S. No.	Assignment of peaks	FTIR of pure whole blood	FTIR of drug injected whole blood	FTIR of drug injected whole blood with Pd(II)
1.	-C-I bonds of blood molecules	558cm^{-1}	664 cm^{-1}	680 cm^{-1}
2.	-C-N bond stretch	Absent	1240 cm^{-1}	1235 cm^{-1}
3.	-C=N bonds of blood proteins	1643 cm^{-1}	1657 cm^{-1}	1657 cm^{-1}
4.	-C=O stretch of drug molecule	Absent	1180 cm^{-1}	Absent
5.	-CH bending of blood molecules	1393 cm^{-1}	1403 cm^{-1}	1403 cm^{-1}
6.	CH_2COO^- stretch of blood	1459 cm-1	1464 cm^{-1}	1474 cm^{-1}
7.	-NH bending of blood molecules	1564 cm^{-1}	1545 cm^{-1}	1555 cm^{-1}
8.	Ar-COO^- stretch of drug molecule	Absent	1321 cm^{-1}	Absent
9.	Pd –O bond stretch of complex	Absent	Absent	502 cm^{-1}
10.	Free –OH stretch	3453 cm^{-1}	3317 cm^{-1}	3424 cm^{-1}
11.	-C-H stretch of blood molecules	2927 cm^{-1}	2930 cm^{-1}	2930 cm^{-1}

TABLE 4: CHARACTERSTIC FTIR BANDS OF PURE SERUM, DRUG INJECTED SERUM AND DRUG INJECTED SERUM WITH Pd(II)

S. No.	Assignment of peaks	FTIR of pure serum	FTIR of drug injected serum	FTIR of drug injected serum with Pd(II)
1.	-CH_2-S- stretch	617 cm^{-1}	675 cm^{-1}	669 cm^{-1}
2.	-C-O- stretch of drug molecule	Absent	1082 cm^{-1}	1053 cm^{-1}
3.	-C=O stretch of drug molecule	Absent	1174 cm^{-1}	Absent
4.	-C=N bonds of blood proteins	1657 cm^{-1}	1673 cm^{-1}	1655 cm^{-1}
5.	-N-H bonds of blood proteins	1551 cm^{-1}	1464 cm^{-1}	1542 cm^{-1}
6.	-CH stretch of blood molecules	2933 cm^{-1}	2920 cm^{-1}	2926 cm^{-1}
7.	-NH bending of blood molecules	1235 cm^{-1}	1240 cm^{-1}	1234 cm^{-1}
8.	-CH bending of blood molecules	1406 cm^{-1}	1403 cm^{-1}	1397 cm^{-1}
9.	Pd –O bond stretch of complex	Absent	Absent	505 cm^{-1}

TABLE 5: CHARACTERSTIC ^{1}H NMR peaks OF PURE SERUM, DRUG INJECTED SERUM AND DRUG INJECTED SERUM WITH Pd(II)

S. No.	Assignment of peaks	1H NMR of pure serum	1H NMR of drug injected serum	1H NMR of drug injected serum with Pd(II)
1.	-R-CH_3 protons of serum	0.8-0.9 ppm	0.9-1.0 ppm	0.9-1.1 ppm
2.	Protons of carbon in vicinity of iodine atom in serum	1.3 ppm	1.31 ppm	1.29 ppm
3.	R-CH_2- protons of serum	1.45ppm	1.45 ppm	1.45 ppm
4.	CH_3-N- protons of drug	Absent	1.89 ppm	1.9 ppm reduced intensity
5.	R-C≡C-H protons of proteins	2.4 ppm	2.0-2.12 ppm	2.0-2.15 ppm
6.	-CH_3 protons of drug molecule	Absent	2.2-2.4 ppm	2.2-2.4 ppm
7.	O-C=R-CH_3 protons of serum	3.2 ppm	2.5-2.7 ppm	2.6-2.8 ppm
8.	CH-C=O proton of drug	Absent	3.2 ppm	Absent
9.	-N-CH_2-CH_2-N- protons of drug	Absent	3.52 -3.63 ppm	3.5-3.8 ppm
10.	Aromatic protons of serum	4.0-4.1 ppm	3.71-4.0 ppm	3.8-4.1 ppm

On the basis of above studies proposed structure of 1:2 complex of Pd(II): diltiazem is as shown in figure I below.

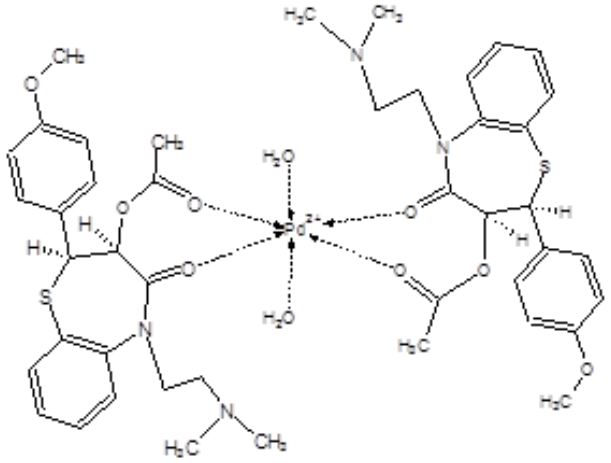

FIGURE I: PROPOSED STRUCTURE OF 1:2 COMPLEX OF Pd(II): DILTIAZEM

Determination of the drug diltiazem can be done by simple calculations using the proposed method. The method evolved is accurate, sensitive and does not need any preparation step, derivatisation or any sophisticated instrumentation.

This method of determination of diltiazem using Pd(II) can be used as routine analysis of pharmaceutical samples and *in-vivo* samples in industrial sectors and pathological labs. Also it can be used for determination of Pd(II) using diltiazem as reagent.

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