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REVIEW OF DILTIAZEM ANALYSIS METHODS DURING 2010-2020

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ABSTRACT

Diltiazem is an anti-cardiovascular drug called calcium channel blocker (CCB), a non-dihydropyridine derivative, which is widely used in the treatment of cardiac ischemia (angina), arrhythmia, and hypertension. Diltiazem preparations on the market are in the form of tablets, Extended-release Capsules, Extended-release tablets, or intravenous injection. This article review discusses the summary of diltiazem analysis methods in various samples that have been used for the past ten years (2010 to 2020). Some of the analytical methods reported are UV / Vis spectrophotometry, spectrofluorimetry, high-performance liquid chromatography (HPLC), voltammetry, electrophoresis, and flow analysis. The HPLC method is the most widely applied analytical technique in the last ten years (2010-2020).

KEYWORDS: Diltiazem; spectrophotometry; voltammetry; HPLC; pharmaceutical preparations; biological liquid samples.

INTRODUCTION

Diltiazem is classified as a calcium channel blocker (CCB) which is widely used in the management of cardiovascular diseases such as ischemia (angina), arrhythmias, and hypertension. CCB works by competitively blocking calcium channels with their agonists, thereby reducing the amount of extracellular calcium that enters the cell.[1] Diltiazem HCl is a diltiazem preparation that is available on the market. It has the chemical name (+) - 5 [2-(Dimethyl-amino) ethyl] -cis-2,3-dihydro-3-hydroxy-2- (p-methoxyphenyl) -1,5-benzothiazepine 4 (5H) -on acetate (ester) monohydrochloride, with the chemical formula $C_{22}H_{26}N_2O_4S.HCl$ (Figure 1) and a molecular weight of 450.98. It is in the form of crystal powder or small crystal, white, odorless, and fused at 210 °C with decomposition. It has solubility that is easily soluble in chloroform, methanol, formic acid, and water, rather

difficult to dissolve in absolute ethanol, and insoluble in ether.[2] The partition coefficient value in octanol/water is 2.79.[3]

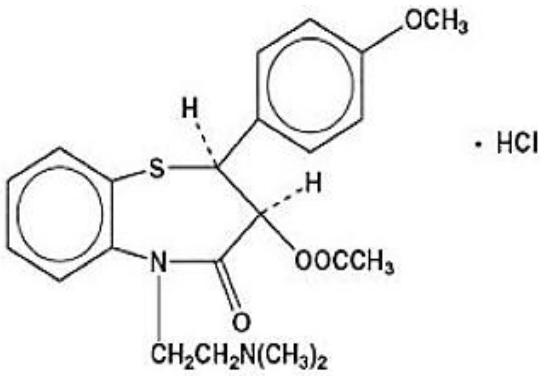

Figure 1: Structure formula of diltiazem hydrochloride.[2]

Based on the pharmacokinetic data obtained, absorption of diltiazem through the gastrointestinal tract is rapid and nearly perfect, 80-90% of the initial dose after oral administration, but experiences extensive first-pass hepatic metabolism with a low bioavailability value of around 30 – 40 %. Peak plasma concentrations are reached after 2 - 4 hours of oral administration. This drug is distributed to body tissues quickly and extensively, about 2 – 4 % of the dose is excreted in the urine in an unchanging form and the rest of its metabolites are excreted in bile, feces, and can be found in breast milk. This drug is metabolized mainly in the liver into several active and inactive metabolites by the cytochrome P450 CYP3A4 isoenzyme.[3,4]

Several methods have been reported related to diltiazem analysis both in pure compounds or pharmaceutical preparations and in biological fluids. Until now, many analytical methods have been developed for the determination of the levels of diltiazem which will be summarized from the data of the last ten years (2010 - 2020).

DATA COLLECTION

Searching for data in this article review is done by collecting data from sources and literature in the form of pharmaceutical books and online research journals on the internet through trusted sites such as Google Scholar, ScienceDirect, and NCBI with the search keywords "Determination of Diltiazem", "Diltiazem Hydrochloride", “Diltiazem Analysis". Primary data were obtained from international journals with inclusion criteria, namely journals published from 2010 to 2020, discussing the analytical methods used to determine the levels of the drug diltiazem that have been used for the past ten years.

DILTIAZEM ANALYSIS METHODS

Ultraviolet spectrophotometric analysis

Several ultraviolet spectrophotometric analysis methods have been used in determining the levels of diltiazem in pharmaceutical preparations (Table 1).

Table 1: Analysis of diltiazem using ultraviolet spectrophotometry.

No.	Sample	Solvent	Wavelength	Range of Concentration	Ref.
1.	Bulk, tablet dosage	Distilled water	226-246 nm (λ_{max} 236 nm)	6-16 µg/ml	[5]
2.	Pure, tablet dosage forms	0.1 M HCl	238 nm	2-25 µg/ml	[6]
3.	Bulk, pharmaceutical formulation	0.1 N HCL (hydrochloric buffer)	237 nm	5-15 µg/mL	[7]

A paper describes the development and validation of UV spectrophotometric methods for estimating diltiazem hydrochloride in tablet formulations using the area under the curve method. Standard solutions and samples of diltiazem hydrochloride are prepared in distilled water. The area under the curve between 226 to 246 nm is measured. This method follows linearity in the range of 6 - 16 µg/mL with a correlation coefficient value of 0.999. This method is validated for various parameters according to ICH Q2 (R1) guidelines. The standard deviation value is satisfying. The relative percentages for intra-day and inter-day precision indicate that the method is appropriate. The percentage of recovery study was found to be 105.69%. The detection limit and the quantitation limit obtained were 0.3197 µg/mL and 0.9689 µg/mL, respectively.[5]

A new, fast, sensitive, economical, and simple spectrophotometric method has been developed for the determination of diltiazem hydrochloride (DT-HCl) in tablets and pure dosage forms. In this method, simpler direct spectrophotometric measurements in the ultraviolet region have been developed for the determination of DT-Cl without any chemical reagents. A DT-HCl solution in 0.1 M HCl shows maximum absorbance at 238 nm. After optimization, the system complies with Beer's law in the concentration range of 2-25 µg/mL. The apparent molar absorptivity was found to be 1,837 x 104 L.mol $^{-1}$.cm^{-1}. In this method, Sandell's sensitivity, slope, intercept, correlation coefficient, relative standard deviation (RSD), the limit of detection, and quantization are also calculated (n = 5). No interference was observed from general excipients present in pharmaceutical formulations. The results are

compared with those obtained by the reference method using the t-test and the F-test. Therefore, this method is suitable for the determination of these drugs, because they are sensitive and precise to some extent.[6]

A study described the development of spectrophotometric methods and the validation of diltiazem hydrochloride and their formulations with greater precision and accuracy. Spectrophotometric measurements were carried out using a JASCO double beam (model V-530) UV-visible spectrophotometer with a pair of quartz cells that match 1 mm and 0.1 N HCl as a hydrochloride buffer. The maximum wavelength is 237 nm. Linearity was observed in the concentration range of 5-15 µg/mL. The percentage of recovery of diltiazem hydrochloride is in the range of 98.9 - 101.7 %. Precision found to be no more than 2.0 % RSD (relative standard deviation). The proposed method has been validated according to the guidelines of the International Conference on Harmonization (ICH). Validation studies show this method is simple, precise, accurate, specific, fast, reliable, and reproducible.[7]

Visible spectrophotometric analysis

Several visible spectrophotometric methods have been used to quantitatively analyze diltiazem levels (Table 2).

Table 2: Diltiazem analysis using visible spectrophotometry.

No.	Sample	Reagent	Wavelength (nm)	Range of Concentration	Ref.
1	Tablet, capsule	Copper (II) in a buffer solution of pH 7 and 4,4'-dicarboxylic-2,2'-biquinoline acid in a micellar medium	558	20 - 100 µg/mL	[8]
2	Tablet, capsule, bulk	4-chloro-7-nitrobenzofurazan (NBD-Cl)	481	16 - 96 µg/mL	[9]
3	Bulk, tablets, biological fluids	bromocresol purple (BCP)	399	2.26 - 27.06 µg/mL	[10]
4	Bulk, tablets, biological fluids	chlorophenol red (CPR)	402	2.26 - 48.48 µg/mL	[10]
5	Bulk, tablet	phenol red (PR)	390	11.28 - 112.75 µg/mL	[6]
6	Bulk, tablet	Chlorophenol red (CPR)	402	2.26 - 48.48 µg/mL	[6]
7	Pharmaceutical formulation	Sulfochlorophenol-S	600	200 - 700 µg/mL	[11]
8	Pharmaceutical formulation	Bromopyrogallol red	440	200 - 700 µg/mL	[11]
9	Pharmaceutical	Eriochromecyanine- R	462	50 - 150	[11]

	formulation			µg/mL	
10	Pharmaceutical formulation	Pyrocatechol violet	442	200 - 500 µg/mL	[11]
11	Pharmaceutical formulation	Palladium(II) Chloride	400	3.413×10^{1} - 2.722×10^{2} µg/mL	[12]
12	Human whole blood	Palladium(II) Chloride	400	1.01×10^{2} - 2.77×10^{2} µg/mL	[12]
13	Human serum	Palladium(II) Chloride	400	1.79×10^{2} - 2.47×10^{2} µg/mL	[12]

A simple method was developed to determine the amount of diltiazem, based on the reduction of copper (II) in a buffer solution (pH 7.0) and the use of micelle media containing 4,4-dicarboxylic-2,2-biquinoline acid. The copper (I) produced reacts with 4,4'-dicarboxylic-2,2 'biquinoline acid and the complex formed is measured spectrophotometrically at 558 nm. A typical calibration graph shows good linearity (r = 0.993) from 20 to 100 mg/mL diltiazem. The detection limit and relative standard deviation were calculated as 12 mg/mL (99 % confidence level) and 3.5 % (40 mg/mL; n = 6), respectively, with an average recovery value of 96.5 % found in pharmaceutical dosages.[8]

A simple, sensitive, accurate, and inexpensive spectrophotometric method was developed to determine the levels of diltiazem HCl using 4-chloro-7-nitrobenzofurazan (NBD-Cl), both in pure form and in the pharmaceutical dosage form. In this study, this drug reacts with NBD-Cl in the presence of borate buffer pH = 7.6 at a fixed time of 30 minutes in a thermostated water bath at 75-80 °C. Absorbance was measured using a spectrophotometric technique at 481 nm. Linear calibration curves are in the range of 16 - 96 µg/mL when using the spectrophotometric method. The quantization limit and the detection limit are also calculated. This method was successfully applied to commercial dosage forms and can be further applied for large-scale determination in quality control laboratories. The results obtained are statistically by those obtained by the reference method.[9]

Two fast, simple, precise, and sensitive extractive spectrophotometry methods (A and B) have been developed for the determination of the levels of diltiazem hydrochloride in the form of pure drugs, tablet preparations, and biological fluids. Both methods (A and B) involve the formation of an intense yellow ion association complex between the drug and one of the purple bromocresol reagents (BCP) or red chlorophenol (CPR) followed by extraction

with methylene chloride. The ion association showed maximum absorption at 399 and 402 nm with BCP and CPR. The calibration curve resulting from measuring the absorbance-concentration relationship (under optimal reaction conditions) of the extracted ion association complex is linear in the concentration range of 2.26-27.06 and 2.26-48.48 µg / mL respectively with BCP and CPR. Molar absorptivity and Sandell's sensitivity from the product reaction were calculated. In methods A and B slope, intercept, correlation coefficient, relative standard deviation (RSD), the limit of detection, and quantization are also calculated (n = 5). No interference was observed from general excipients present in pharmaceutical formulations. The results obtained are compared with the reference method using the t-test and F test. Therefore, these methods are suitable for drug determination, because they are sensitive and precise to a certain extent.[10]

Two new, fast, sensitive, economical, and simple spectrophotometric methods B and C have been developed for the determination of micro diltiazem hydrochloride (DT-HCl) in tablets and pure dosage forms. Methods B and C involve the formation of a complex of intense yellow ion associations between this drug and one of the phenol red (PR) or chlorophenol red (CPR) reagents followed by extraction with methylene chloride. Ion-associates shows maximum absorption at 390 and 402 nm for PR and CPR, respectively. After optimization, the system obeys Beer's law in the concentration range of 11.28 - 112.75 and 2.26 - 48.48 µg/mL with PR and CPR, respectively. The molar absorptivities seen were 4,086 x 10^3 and 9,919 x 10^3 L mol-1.cm^{-1} with PR and CPR, respectively. In this method, Sandell's sensitivity, slope, intercept, correlation coefficient, relative standard deviation (RSD), the limit of detection, and quantization are also calculated (n = 5). No interference was observed from general excipients present in pharmaceutical formulations. The results are compared with those obtained by the reference method using the t-test and the F-test. Therefore, this method is suitable for the determination of these drugs, because they are sensitive and precise to some extent.[6]

Four simple, sensitive, and accurate spectrophotometric methods have been developed to determine calcium channel blocker drugs: Diltiazem hydrochloride in pharmaceutical formulations. These methods are based on complex ion-pair formation with Sulfochlorophenol-S (SCPS), Bromopyrogallol red (BPR), Eriochromecyanine-R (ECC) and Pyrocatechol violet (PCV) in acidic medium. The colored product was extracted with chloroform and measured spectrophotometrically at 462, 600, 440, and 442 nm for ECC,

SCPS, BPR, and PCV, respectively. Based on beer's low rules, the concentration ranges of diltiazem are 50 - 150, 200 - 700, 200 - 700, and 200 - 500 µg/mL for ECC, SCPS, BPR, and PCV, respectively, with molar absorptivity for ECC, SCPS, BPR , and PCV respectively are 2.1×10^4, 5.6×10^3, 4.8×10^3, 19.5×10^3, 6.6×10^3 L/mol/cm, and the relative standard deviation is 0.86 %, 0.96 %, 0.95 %, and 0.65 % for ECC, SCPS, BPR, and PCV, respectively. These methods have been successfully applied for testing these drugs in pharmaceutical formulations. No disturbances were observed from common pharmaceutical adjuvants. A comparison of statistical results with the reference method shows a very good agreement and shows no significant differences in accuracy and precision.[11]

Analytical methods have been developed for the quantitative determination of diltiazem through complexation with Pd (II) by spectrophotometry. Diltiazem forms a stable orange 1 : 2 complex with palladium (II) chloride with λ_{max} 400 nm, complex molar absorptivity coefficient $\varepsilon = 8.5 \times 10^2$ L/mol/cm, Beer legal range $3{,}413 \times 10^1$ µg/mL to $2{,}722 \times 10^2$ µg/mL with a regression interception of 0.019 and a correlation coefficient of 0.989. Disruption of foreign metal ions and the effect of temperature and pH were also studied. Complex characterization involves Element Analysis, FTIR, ^{1}H NMR, ESR, Raman spectrum, magnetic susceptibility measurement, and thermal studies. Based on studies above complex structures have been proposed. The analytical method developed was applied to blood samples in-vivo whole blood and serum samples. For complete blood samples, Beer's legal range is 1.01×10^2 µg/mL to 2.77×10^2 µg/mL with variance coefficient ± 1.49 and relative standard deviation of 0.64 % and for serum samples Beer's legal range is 1.79×10^2 to 2.47×10^2 µg/mL with variance coefficient ± 1.03 and relative standard deviation of 0.79 %. This procedure is fast, accurate with precision, and can be used by pathologists and in the industrial sector for the determination and quality testing of diltiazem in pharmaceutical samples.[12]

Spectrofluorimetric analysis

A simple, sensitive, accurate, and inexpensive spectrofluorimetric method was developed for the determination of diltiazem HCl using 4-chloro-7-nitrobenzofurazan (NBD-Cl) accompanied by kinetic studies, both in pure form and in pharmaceutical preparations. Based on this study, the drug was reacted with NBD-Cl at pH 7.6 by borate buffer at a fixed time of 30 minutes on a thermostated water bath at 75 - 80 °C. Absorbance is measured after dilution at certain wavelengths of excitation and emission. Linear calibration curves in the range of

1.6 - 8.8 µg/mL. The quantization limit and the detection limit are also calculated. This method was successfully applied to commercial dosage forms and can be further applied for large-scale determination in quality control laboratories. The results obtained statistically agree with those obtained by the reference method. Determination of the drug being investigated with the rate concentration is feasible with the calibration equation obtained.[9]

Analysis of high-performance liquid chromatography (HPLC)

Many high-performance liquid chromatography methods that have been used quantitatively to analyze diltiazem levels are summarized as follows (Table 3).

Table 3: Analysis of diltiazem using HPLC.

No.	Sample	Column	Mobile phase	Detector	Chromatographic Condition	Ref.
1.	Bulk, tablet	3,9 mm x 30 cm column	Buffer (1.16 g d-10-camfersulfonic acid P in 1000 ml of 0.1 M sodium acetate); acetonitrile P methanol P (50:25:25 v/v/v, pH 2.6)	UV 240 nm	Flow rate: 1.6 mL/min	[2]
2.	Raw materials, tablets, human serum	Hiber®, 250-4.6 RP-18 column	Acetonitrile-water (85 : 15 v/v, pH 2.6 ± 0.02)	UV/visible detector (230 nm)	Flow rate: 1.0 mL/min Temperature: Ambient	[13]
3.	Tablet dosage form, bulk, human serum	Column C_{18}	methanol-water (80 : 20 v/v, pH 3.1 ± 0.02)	Not available	Flow rate: begin 0.5 mL/min, then increased to 1 mL/min Temperature: Ambient	[14]
4.	Raw materials, tablets, human serum	Hiber, 250-4.6 RP-18 column	acetonitrile–methanol–water (30:20:50, v/v, pH 2.59 ± 0.02)	UV–vis detector (230 nm)	Flow rate: 1.0 mL/min Temperature: Ambient	[15]
5.	Tablet	Hypersil BDS C18 (150 mm x 4.6 mm, 5.0 µm)	0.2% Triethylamine (TEA) in combination with acetonitrile (ACN)	UV 240 nm	Flow rate: 1.0 mL/min	[16]
6.	Tablet	Inertsil ODS-3, 5 µm (4.6 x 250 mm) column	Buffer –acetonitrile – methanol (500:250:250 v/v, pH 6.2 ± 0.05)	ultraviolet detector (240 nm)	Flow rate: 1.6 mL/min	[17]
7.	Gel (topical)	C_{18} analytical column	ethanol: phosphoric acid (35 : 65, v/v, pH 2.5)	UV detector (240 nm)	Flow rate: 2.0 mL/min Temperature: 50 ± 1 ^{o}C	[18]
8.	Human serum	Pinnacle II Cyano column	4.15 $\times 10^{-2}$ mol/L sodium dodecylsulfate dan 0.02	UV 225 nm	Flow rate: 0.8 mL/min	[19]

			mol/L sodium dihydrogen phosphate, dengan 10% (v/v) 1-propanol, pH 7.0)		Temperature: 40 ^{o}C.	
9.	Human plasma	Zorbax SB-C18 column, (4.6 mm x 250 mm, 5 µm) is equipped with a refillable guard column (30-40 µm)	0.2 M ammonium dihydrogen phosphate: acetonitrile: isopropyl alcohol: triethylamine (55:43:1.7:0.3, v/v, pH 4.5)	UV detector (240 nm)	Flow rate: 0.7 mL/min	[20]
10.	bulk, tablet, human serum	Pruospher® Star RP-18 endcapped (250 mm x 4.6 mm id)	Acetonitrile: methanol: water (5:45:50 v/v, pH 2.5)	UV Detector (230 nm)	Flow rate: 1 mL/ min Temeparure: room 25 ^{o}C	[21]
11.	Tablet, bulk	RP C-18 column	0.01 M ammonium acetate in water: methanol: acetonitrile (700: 240: 60 v / v)	photodiode array detector (295 nm)	Flow rate: 1 mL/min	[22]
12.	Bulk, tablet, human serum	Nuclosil 100-10 C-18 (250×4.6mm)	acetonitrile: methanol: water: (10:55:35 v/v, pH 2.65 ± 0.02)	UV/visible detector (240 nm)	Flow rate: 1 mL/ min	[23]
13.	Tablet, human plasma	MZ-analytical column (15 mm × 4.6 mm, 5 µm)	acetonitrile/2-propanol/15 mM phosphate buffer (pH = 2 ± 0.05) (32.5/2.5/65 v/v/v)	UV detector (225 nm)	Flow rate: 0.9 mL/min Temperature: 25.0 ± 0.5 °C	[24]
14.	Pure drug	Kromasil C18 column (300 mm x 4 mm i.d)	methanol: water (90:10: v/v)	UV detector (237 nm)	Flow rate: 1 mL/min	[25]
15.	Rat plasma	C18 column coated with 5 micron particles	Acetonitrile: Water: Ortho phosphoric acid (65:35:0.1% v/v, pH 2.8)	UV detector (235 nm)	Flow rate: 1 mL/min Temperature: room	[26]
16.	Human serum, clinical laboratories, and pharmaceutical formulations	Purospher Star, C18 (5 µm, 25 x 0.46 cm) column	methanol-water (80:20 v/v, pH 3.4)	ultraviolet detector (220 nm)	Flow rate: 1.0 mL/min Temperature: Ambient	[27]
17.	Pure drugs in wastewater samples at ultra-trace levels	Agilent, Zorbax, SB-C8 (100 mm×2.1 mm× 3.5 µm)	The mobile phase A (0.10% formic acid + 5.0 mM ammonium formate in ultrapure water) and the mobile phase B (0.10% formic acid + 5.0 mM ammonium formate in methanol)	6410A type quadrupole MS detector	Flow rate: 0.5 mL/min	[28]
18.	analytical grade in domestic wastewater treatment plants	Agilent, Zorbax, SB-C8 (100 mm×2.1 mm× 3.5 µm)	0.1% formic acid + 5.0 mM ammonium formate in H_2O (Mobile Phase A) ultrapure and 0.1% formic acid + 5.0 mM ammonium formate in CH_3OH for	6410A type quadrupole MS detector	Flow rate: 0.5 mL/min	[29]

	(Influent and effluent samples)		(Mobile Phase B)			
19.	Active pharmaceutical ingredients, dosage formulations, human serum	Nucleosil® C18 (10 µm, 25 × 0.46 cm) column	methanol: water: acetonitrile (55:35:10 v/v; pH 2.65)	UV detector (238 nm)	Flow rate: 1.0 mL/min Temperature: Ambient	[30]
20.	Bulk, pharmaceutical dosage form	Zorbax [C8 (5µm, 4.6mm ×250)]	buffer and acetonitrile in the ratio of (60:40)	UV 240 nm	Flow rate: 1.0 mL/min	[31]
21.	Pasta, bases, salt	XBridge C18 (4.6 mm 250 mm, 5 µm)	Acetonitrile: ammonium formate 0.05 M (pH 3.1)	DAD detector (274 nm)	Flow rate: 1.0 mL/min Temperature: 60^{o}C	[32]
22.	Human plasma	Agilent ZORBAX Eclipse column (4.6 mm × 100 mm, 3.5 Mm)	0.02 M phosphate buffer (pH 3.8) ; acetonitrile (65: 35, v/v)	UV detector (200 nm)	Flow rate: 1.0 mL/min	[33]
23.	Human urine	Column C18 extended (250 x 4.6 mm; 5 mm; 80 Å)	Acetonitrile-trifluoroacetic acid 0.026 % (v/v; pH 2.5)	PDA detector (235 nm)	Flow rate: 1.0 mL/min Temperature: 25 ± 2^{o}C	[34]
24.	Sludge samples	Agilent, Zorbax, SB-C8 (100 mm x 2.1 mm x 3.5 mm)	0.1 % formic acid + 5.0 mM ammonium format in ultrapure H_2O (mobile phase A), dan 0.1 % formic acid + 5.0 mM ammonium formate in CH_3OH (mobile phase B)	6410A type quadrupole MS detector	Flow rate: 0.2 mL/min	[35]

The determination of diltiazem levels in tablets/powder is carried out as follows. Make a test solution by weighing carefully and powdering no less than 20 tablets or the equivalent of approximately 600 mg of diltiazem hydrochloride, then put in a 500 ml measuring flask. Add 200 ml of methanol P, sonication for 1 hour then chill, dilute with methanol P until the mark. Centrifuge 25 ml of liquid at 3500 rpm for 15 minutes and inject the liquid into the chromatograph. The high-performance liquid chromatograph is equipped with a 240 nm detector and a 3.9 mm x 30 cm column containing L1 fillers. The flow rate of approximately 1.6 mL per minute. The relative retention time of diltiazem is 1.0, and the number of theoretical plates at the peak of diltiazem is not less than 1200. The relative standard deviation of re-injection is no more than 2.0 %. Procedure: Calculate the amount in mg, diltiazem hydrochloride, $C_{22}H_{26}N_2O_4S.HCl$, in tablet powder used with the formula:

$$500.C = r_u/r_s$$

C is the level of Diltiazem Hydrochloride BPFI in mg per mL standard solution. r_u and r_s are respectively the peaks of the test solution and the standard solution.[2]

A high-performance liquid chromatography (HPLC) method has been developed and validated for the simultaneous determination of diltiazem in raw materials, pharmaceutical formulations, and human serum. In HPLC, diltiazem uses acetonitrile-water (85: 15 v/v, pH 2.6 ± 0.02) as a mobile phase at a flow rate of 1.0 mL/minute at ambient temperature. The separation was performed on the Hiber column, 250-4.6 RP-18, equipped with a UV/visible detector at 230 nm. This method has been successfully applied to pharmaceutical formulations because no chromatographic disorders from tablet excipients were found. Linearity was found to be in the range of 0.625-20 mg/mL. The suitability of the method for the quantitative determination of drugs is proven by validation by the requirements established by the International Conference on Harmonization (ICH) guidelines. The results of the validation, together with handling statistics from the data, show the reliability of this method.[13]

Simple, precise, accurate, selective, and sensitive reversed-phase LC-UV methods have been developed for the simultaneous analysis of diltiazem in bulk drugs, tablet dosage forms, and human serum. The chromatographic separation of the drug was carried out at room temperature in the stationary phase C18 with 80:20 (v / v) methanol-water, pH 3.1 ± 0.02, as the isocratic mobile phase. The mobile phase flow rate is initially 0.5 mL/min and then increased to 1 mL/min, until all are separated from each other. The total period is 10 minutes. This test was successfully applied to pharmaceutical and serum formulations and there were no chromatographic disorders from tablet excipients. This method is linear in the range of 1.25 - 50 µg/mL. The suitability of this HPLC method for quantitative analysis of this drug is proven by validation that is by International Conference on Harmonization (ICH) guidelines. The results of the validation, and statistical analysis of the data, show this method is reliable.[14]

An inexpensive, simple, and stable stability RP-HPLC method was developed for the determination of diltiazem in topical preparations. Separation is based on the analytical column C18 using a mobile phase consisting of ethanol: phosphoric acid solution (pH = 2.5) (35: 65, v/v). The column temperature is set at 50 ^{o}C and quantization is achieved by UV detection at 240 nm. In forced degradation studies, the drug underwent oxidation, hydrolysis,

photolysis, and heat. This method is validated for specificity, selectivity, linearity, precision, accuracy, and robustness. The procedure applied was linear in the diltiazem concentration range of 0.5 - 50 µg/mL (r^2 = 0.9996). The precision was evaluated by a repeat analysis where the % relative standard deviation (RSD) value for the area was found to be below 2.0. Recovery value obtained (99.25 % - 101.66 %) ensures the accuracy of the method developed. Degradation products and pharmaceutical excipients are well-resolved from pure drugs. The expanded precision (5.63 %) of this method is also estimated from the method validation data. Thus, the proposed validated and ongoing procedures proved to be suitable for routine analysis and studies of the stability of diltiazem in pharmaceutical preparations.[18]

A micellar per aqueous Liquid Chromatographic method are investigated simultaneously to determine diltiazem hydrochloride in human serum. Separation and determination of the analyte were carried out in the Pinnacle II Cyano column as a stationary phase, using a mobile phase consisting of a dilute solution (4.15×10^{-2} mol/L sodium dodecyl sulfate and 0.02 mol/L sodium dihydrogen phosphate) with 10 % (v/v) 1-propanol at pH 7.0. This method is validated by linearity, the lower limit of quantification, recovery extraction, stability, precision, and accuracy. The main analytical parameters are linearity (r > 0.9950), intra and inter-day precision (RSD intra-day 2.2 - 3.5 %, and RSD inter-day 3.7 - 9.5 %), the limit of quantification is lower 20 µg/mL. Extraction recovery was 67.1 % (0.1 µg/mL), 68.8 % (1.0 µg/mL) and 73.8 % (2.5 µg/mL). The relative stability error is < 6.4 % at room temperature for 24 hours, < 3.8 % at 4 ^{o}C for 1 week, < 4.6 % at -20 ^{o}C for 1 month, and < 6.7 % for the freezing/thawing cycle (n = 3). The results show that the proposed method is fast, sensitive, and accurate for the determination of levels of human serum diltiazem.[19]

A new analytical method for determining diltiazem was developed through liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS-MS / MS) after pre-concentration with solid-phase extraction (SPE). Influent and effluent samples taken from five different wastewater treatment plants throughout Turkey, namely Hurma/Antalya, Lara/Antalya, Kemer-1, Kemer-2, and METU / Ankara were analyzed to determine the levels of diltiazem under optimal conditions. All parameters in the pre-concentration step were optimized and recovery was best at pH 7 (around 100 %). Diltiazem was found in all samples of temporary treatment plants except for Lara/Antalya waste.[29]

The determination of diltiazem levels was carried out using HPLC-electrospray tandem MS. The sequential extraction of ultrasound-assisted sludge samples was optimized to increase the

efficiency of analyte extraction; ranging from 93.0 to 98.3 % recovery value. The limit of detection value was found at 0.78 μg/kg. Mud samples were taken from Ankara Tatlar; Hurma, Lara and Kemer Antalya, and the Middle East-Technical University of vacuum-rotating wastewater treatment plant (WWTP) membranes for analysis of diltiazem levels under optimized conditions. Diltiazem was found in all samples in the range between 116.4-180.8 μg/g.[35]

Electrochemistry analysis

Several electrochemical methods have been used for quantitative analysis of diltiazem levels. The following will explain some of the electrochemical based diltiazem analysis methods.

In this study, a novel application of bismuth film electrodes (BiFEs) was developed for the determination of antihypertensive and coronary vasodilators by square-wave cathodic voltammetry. The bismuth film was deposited externally on a glass carbon electrode for 90 seconds at -1.4 V vs. Ag/AgCl, from an acetate buffer (pH 4.5; 0.10 mol/L) containing 5 or 30 mg/L Bi. The analytical diltiazem signal is obtained in phosphate buffer (pH 7.4; 0.25 mol/L) where the reduction occurs at -1.5 V vs. Ag/AgCl. The proposed methodology is applied to the quantification of diltiazem in pharmaceutical samples (dynamic linear range consisting of 90 and 900 μg/L) and in human urine (dynamic linear range consists of 45 and 270 μg/L, and the detection limit is 12 μg/L).[36]

A chemically modified electrode is made based on carbon electrodes modified with modified graphene oxide (nanoFe_3O_4@GO-GC). Initially, the electrodes were evaluated as electrochemical sensors for the simultaneous determination of diltiazem and timolol in aqueous solutions. Measurements were made by applying the differential pulse voltammetry method in a phosphate buffer solution with a pH of 6.00. The results showed that nanoFe_3O_4@GO increased the oxidation rate by increasing peak currents. nanoFe_3O_4 loaded in GO can increase the anodic peak current from diltiazem and timolol on the electrode surface. Electrostatic interactions between the diltiazem cation and timolol and the high electron density of the nanoFe_3O_4@GO hydroxyl group will cause an increase in the diltiazem concentration around the surface of the modified electrode and the peak current increases significantly. The prepared electrodes showed a voltammetric response with good selectivity for diltiazem and timolol in optimal conditions, which made it very suitable for the simultaneous determination of this drug. The practical analytical utility of the modified

electrode is illustrated by the simultaneous determination of diltiazem in thorny serum samples.[37]

A study described the electrochemical activity of gold nanoparticles covered in dodecanethiol (AuNPs) obtained by the modified biphasic method. Stoichiometric ratio variation between [$AuCl_4^-$] and dodecanethiol has produced nanostructures of different sizes and different electrochemical activities. Using an Au: dodecanethiol (Au: Thiol) molar ratio of 1: 1, 2: 1 and 4: 1, gold nanoparticles were obtained with an average diameter of 2.3 ± 0.7; 3.1 ± 0.5 and 4.3 ± 0.7 nm. A cyclic voltammetry experiment (CV) using glass carbon electrodes modified with gold nanoparticles was carried out and the effect of the amount of AuNP, pH, scanning stroke, and current stability on the voltammetry response was analyzed. The system that has presented the best electroactivity for oxide formation is evaluated as a sensor for the determination of Diltiazem (DTZ). The voltammetry response was observed in the presence of DTZ which was associated with the electrochemical-chemical (EC') mechanism between the analyte and the electrode surface. Cathodic peak currents obtained from Linear Sweep Voltammetry (LSV) are reduced linearly with increasing DTZ concentrations, obtaining linear profiles for DTZ solutions ranging from 4.0 to 13.5 nmol/L with detection limits and quantification limits of 0.5 and 1.7 nmol/L, respectively.[38]

An anodic stripping voltammetry method for the determination of nanomolar diltiazem with chemically modified carbon paste electrodes (CMCPE) containing Co_3O_4/SnO_2 nanopowders was studied. The potential accumulation and time were chosen, -0.2 V and 190 s, respectively. The CMCPE electroanalytic performance was evaluated regarding the composition of the carbon paste, the pH of the solution, the time and potential for accumulation, and the potential for interference. Novel electrodes showed a linear response to the concentration range of diltiazem 50 - 650 nM with the lowest detection limit value of 15 nM. The precision for the six determinations of 350 and 550 nM diltiazem was 3.2 and 2.5 %, respectively. That shows that the proposed method is free from most disturbances. Finally, this method is effectively applied to the determination of diltiazem in pharmaceutical tablets and biological samples.[39]

In this paper, diltiazem hydrochloride (DTZ) is determined electrochemically on a cathodic doped diamond electrode. The sustainable method shows potential applications using two supporting electrolytes, evaluating two different drug oxidation peaks. For the determination of DTZ voltammetry, all operating parameters of square wave voltammetry are optimized

and an analytical curve is built. The in-house voltammetry method is validated in terms of linearity, detection limits, quantification limits, precision, accuracy, and selectivity. Pharmaceutical samples were analyzed by official methods and results were statistically similar to those obtained by the voltammetry method. A truly runny voltammetry procedure is also applied to the determination of DTZ in biological fluids with excellent recovery values.[40]

A Montmorillonite-ZnO (MMt/ZnO) micro-hybrid sensor which has a hierarchy like 3D has been successfully created as a highly sensitive electrochemical sensor to detect Diltiazem hydrochloride (DZM.HCl). Zn-like 3D hierarchies such as flowers and MMt/ZnO hybrid series have been synthesized using different MMt [FMZ1-5] content through the polymer hydrogel template method using alginate ions. The effect of combining various MMt content on morphology, the hybrid surface area was investigated using Fourier transform infrared (FTIR), X-ray diffraction (XRD), scanning field emission electron microscopy (FE-SEM), Energy-dispersive X-ray spectroscopy (EDS), Brunauer-Emmett-Teller (BET) surface area method, and high-resolution transmission electron microscope (HR-TEM). Hybrids [FMZ3] obtained with 2.0 % MMt produce the most perfect flower-like morphology and highest surface area (190.06 m^2/g) with the lowest resistivity. The hierarchical structure [FMZ3] reveals the ZnO nanosphere with an average diameter of 5.49 nm, arranged into nanorods followed by assembling to form flower-like shapes with the inclusion of MMT layers and peeled in stems with spacing ranges from 1.1 - 7.4 nm. Meanwhile, the modified sensor applied 1.0 % [FMZ3] which is modified by CPS maintains excellent conductivity and electrocatalytic activity as assessed from cyclic voltammetry (CV) measurements. As a result, an investigation of the electrochemical behavior and oxidation mechanism of the DZM.HCl drug on the sensor surface was built. Under optimal operational conditions, the proposed sensor managed to reach the detection limits of 0.177, and 0.21 nmol/L from DZM.HCl in commercial and human biological fluids (serum samples, respectively). The built sensors produce accurate accuracy and are free from obstacles from other ordinary drug excipients.[41]

Electrophoresis analysis

The electrophoresis method has been used for quantitative diltiazem analysis. Some of these methods will be discussed below.

A simple, sensitive, and selective determination of diltiazem hydrochloride (DLT) is explained using electrochemiluminescence capillary electrophoresis (CE-ECL). CE-ECL

parameters that affect separation and detection are optimized. Under optimized conditions, the linear range of DLT is from 0.02 to 100 mol/L (r^2 = 0.9983), with a detection limit of 5.1 nmol/L. The relative standard deviation from ECL intensity and migration time is < 2 % for 0.1 mol/L and 22 mol/L DLT (n = 11). A new technique for determining the number of binding sites and binding constants between DLT and human serum albumin (HAS) was developed using ultrasonic microdialysis coupled with CE-ECL. The number of binding sites and binding constants are 5.9 and 6.3 × 10^4 L/mol, respectively. The time required for ultrasonic microdialysis is 10 times less than for traditional dialysis. Compared to traditional dialysis, ultrasonic microdialysis is simple, fast, and must apply to a variety of drug and biomacromolecular interactions.[42]

Flow analysis

A flow analysis method has been used to quantitatively determine diltiazem levels. The following will explain the diltiazem analysis method based on flow analysis techniques.

Liquid-liquid microextraction without phase segmentation is implemented in a multicommuted flow system for the determination of anti-hypertensive diltiazem. This procedure is based on the formation of ion pairs between the drug and the bromothymol blue dye at pH 3.5. Detection is carried out without phase separation in a glass tube combined with an optical fiber spectrophotometer. The total volume of chloroform was reduced to 50 µL compared to 10 mL consumed in batches. Linear responses were observed between 9 and 120 µmol/L, with a detection limit of 0.9 µmol/L (confidence level of 99.7 %). The coefficient of variation (n = 10), the sampling rate, and extraction efficiency are each estimated at 0.6 %, 78 determinations per hour, and 61%, respectively. About 30 µg of bromothymol blue is consumed and the volume of waste is 380 µL per count. Results for pharmaceutical samples were approved with a gain of 98 % at the confidence level of the reference procedure.[43]

CONCLUSION

Overall, various analytical methods have been used to estimate diltiazem levels from 2010 to 2020. Spectrophotometry, spectrofluorimetry, voltammetry, electrophoresis, and flow analysis methods are simpler and easier to implement but HPLC analysis is often used in research because it can detect samples with concentration to the nanogram level and is very supportive for biological samples.

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