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Instrumental Analysis of Chlordiazepoxide in Different Matrices

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Abstract: Chlordiazepoxide is considered one of most important sedative and hypnotic benzodiazepines drugs which is currently used all over the world with the increased rate of anxiety drugs. In this literature review, we will introduce the pharmacological action of this drug in addition to most of up-to-date reported methods that have been developed for determination of chlordiazepoxide in its pure form, combined form with other drugs, combined form with degradation products, and in biological samples. Most of the reported analytical methods will focus on spectroscopic, chromatographic and electrochemical techniques.

Keywords: Benzodiazepines, Chlordiazepoxide, Pure form, Degradation products, Biological samples.

Introduction

In 1957, Hoffman La Roche laboratory synthesized chlordiazepoxide (CDZO), the first benzodiazepine, which represent one of the most widespread and primarily classes of drugs used for the treatment of psychiatric disorders, anxiety and insomnia [1, 2]. After two months of laboratory testing, the sedative effect of this compound was confirmed to be introduced and approved by the FDA in 1960 under several trade names as (Libritable and Librium) as capsules, tables and injection [3].

Many structural derivatives of CDZO were synthesized in the following years as for example (oxazepam and diazepam), which approved that they have much stronger sedative effect, and furthermore other benzodiazepines by targeted synthesis, allowed to the production with other characteristic hypnotic and/or anticonvulsant functions, with different biological half-life, to be allowed to a larger extent, and at the same time, problems related to drug abuse were reported [2].

As such, in this review article, CDZO has been studied in respect of chemical characters, mode of action and most reported analytical methods that have been developed for determination of this drug in different matrices.

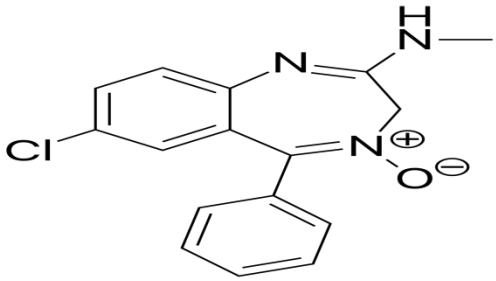

Figure 1. Chemical structure of CDZO

Pharmacological action of CDZO

CDZO is chemically known as 7-chloro-2-methylamino5-phenyl-3H-l,4-benzodiazepine-4-oxide (Figure 1), it is a benzodiazepine class sedative and hypnotic medication showing powerful antianxiety effects in humans used to enhance effectively the effect of the neurotransmitter gamma-amino butyric acid (GABA) complex formation [1,4], which strengthens pharmacological activity of benzodiazepine derivatives related not only to their anxiolytic activity, but also to sedative and hypnotic effect, skeletal muscle relaxant and anticonvulsant activity [2].

However, further study showed, that benzodiazepines intensify the strength of action by acting in binding target sites as agonists by allosteric interaction, increasing GABA binding level, but they have no effect on GABAA receptor activity in the

absence of γ-aminobutyric acid. However, they decrease GABA affinity to GABAA receptor complex with chloride channel, indirectly, increasing frequency, but not the chloride channel opening duration, allowing increased amount of chloride ions penetration under a concentration gradient into the cell [2].

Centrally, Benzodiazepines are used as muscle relaxants without a crucial effect on neuromuscular signaling transduction pathway in motor endplate through the inhibition of multisynaptic impulses [2].

Review of analytical methods

Various techniques were used for the analysis of CDZO in pure forms, in their pharmaceutical formulations and in biological fluids. The available reported methods in the literature can be summarized as follows:

1. Spectroscopic methods

1.1 Spectrophotometric & Spectrofluorimetric methods

Drugs	Matrix	Method or reagent	λ_{max} (nm)	Linearity range	LOD	Ref.
CDZO	Tablets	Indirect method via charge-transfer complex formation with metol	516	3-60 µg/mL	1.16 µg/mL	[1]
CDZO	Tablets	Complexion reaction with palladium chloride	424	5-120 µg/mL	0.20 µg/mL	[3]
CDZO	Tablets	Oxidative coupling reaction with phenothiazine	602	0.1-50µg/mL	0.11 µg/mL	[4]
CDZO	Blood	Combination of ultraviolet spectrophotometry and the Bratton-Marshall reaction	550	0.1-3.0 mg%	-------	[5]
CDZO and clidinium bromide	Tablets	Ratio spectra derivative spectrophotometry	243.1	2.0 - 38.0 µg/mL	-------	[6]
CDZO and Demoxepam	Tablets	Difference spectrophotometry	269	0 - 12.5 µg/mL	-------	[7]
CDZO and Diazepam	Tablets	Combined polynomials method	230-318	0.1-0.7 mg/100mL	-------	[8]
CDZO and Nitrazepam	Tablets	Diazocoupling with ethyl acetoacetate	408	l-16 µg/mL	-------	[9]
CDZO and Amitriptyline HCL	Tablets	Multivariate calibration method	205-305	0.0-4.0 ppm	-------	[10]
CDZO and Mebeverine HCL	Binary Mixture	Novel univariate spectrophotometric methods via different manipulation pathways	200-400	1-12 µg/mL	-------	[11]
CDZO and Trifluoperazine	Tablets	Multicomponent analysis method	245	2-50 µg /mL	-------	[12]
CDZO and 2-amino-s-5-chlorobenzophenone impurity	Degradation product	Fluorimetric method (cerium (IV) sulphatee in phosphoric acid)	λ_{ex} 405 λ_{em} 440 and 465	5 x 10^{-8}- 3 x 10^{-5} g/mL	-------	[13]
CDZO	Pharmaceutical dosage form, urine and plasma	Fluorimetric method (Fluorescamine)	λex 390 λem 486	0.25-6 µg/mL	-------	[14]

2. Chromatographic methods

2.1 HPLC methods

Drugs	Matrix	Column	Mobile phase	Detector	Linearity range	LOD	Ref.
CDZO and mebeverineHCl	Tablets	RP-C_8 (octylsilyl)	Acetonitrile–0.05 M disodium hydrogen phosphate-triethyl amine (50:50:0.2, v/v/v), pH 2.5	UV at 247 nm	2.5–150 µg/mL	0.04 µg/mL	[15]
CDZO	Water, urine, plasma and tablets	MZ ODS- C_{18} (250 × 4.6 mm, 5 µm)	Acetonitrile-water (60:40, v/v)	UV at 254 nm	0.004–10 µg/mL	0.0012 µg/mL	[16]
CDZO	Waste water and tablets	Supelco C_{18} (25 cm x 4.6 mm. 5 µm)	Methanol-acetonitrile- 0.05 MKH^2PO_4 (40: 20 :40 v/v/v)	UV at 246 nm	0.01-0.20 mg/mL	2 µg/mL	[17]
CDZO and Clidinium Bromide	Tablets	Phenomenex Luna C_{18} (250 mm x 4.6 mm, i.d., 5 microm)	Potassium dihydrogen phosphate buffer (0.05 M, pH 4.0adjusted with 0.5% ortho phosphoric acid) methanol– acetonitrile (40:40:20, v/v/v)	UV at 220 nm.	5–500 µg/mL	0.122 µg/mL	[18]
CDZO and diazepam	Urine and plasma	Zorbax SB-C_{18} (Agilent)	Acetonitrile:KH2PO4 buffer solution (5.0 mmol L-1: pH 6.0) (70:30, v/v)	UV at 220 nm	0.005–10.0 µg/mL	0.0012 µg/mL	[19]
CDZO and amitriptyline hydrochloride	Tablets	Waters Spherisorb® C_{18}	Methanol: Acetonitrile:0.2M Orthophosphoric acid, pH 4.5, 56:24:20.	UV at 240 nm	10-320 ng/mL	4.5 ng/mL	[20]
CDZO, diazepam, clonazepam, midazolam, flurazpam, and lorazepam	Soft drinks	C_{18} (250 mm × 4.6 mm, 5µm)	15 mM phosphate buffer: methanol (50:50 v/v)	UV at 245 nm	0.5- 10 µg/mL	0.01 µg/mL	[21]
CDZO, desmethylchlordiazepoxide, demoxepam	Plasma	Hichrom"' $C1_8$ RPB	deionized water/ methanol/ acetonitrile/ acetic acid (51:28:16: 0'2, v:v; pH 3,7)	UV at 241 nm	0,125-10 mg/L	0·075 mg/L	[22]
CDZO and Trifluoperazine HCl	Tablet	Phenomenex (Torrance, CA) C_{18} column	methanol:water (97:03, v/v)	UV at 262 nm	0.5-5 µg/mL	0.05 µg/mL	[23]
CDZO	Tape water, river water, urine and plasma	C_{18} (150 mm, 4.6 mm, 5 µm)	ACN: water (40:60, v/v)	UV at 225 nm	0.006–10 µg/mL	0.0014 µg/mL	[24]
CDZO, diazepam, benzodiazepines	Serum	BondElut C_{18}	35% acetronitrile and 65% 7 mM K_2HPO_4 buffer adjusted to pH 3.7 with 0.1 M phosphoricacid.	UV at 242 nm	50 - 1000 ng/mL	25 ng/mL	[25]
CDZO, Clidinium bromide and dicyclomineHCl	Tablets	Kromasil C_{18}	potassium dihydrogen phosphate buffer (0.05 M, pH 4.0adjusted with 0.5% orthophosphoric acid): methanol: acetonitrile (30:40:30, v/v/v)	UV at 270 nm	24-56 µg/mL	4.58 µg/mL	[26]
CDZO, Flurazepam Oxazepam Lorazepam Clonazepam	Urine Stomach content Tissue Bileand	Knauer 100-5 C_{18} (250 mm × 4.6 mm)	buffer phosphate (pH = 2.32) and acetonitrile (63:37).	PDA	0.1 - 10 µg/mL	0.05 µg/mL	[27]

Alprazolam Diazepam	Other biological samples						
CDZO Anticonvulsants, xanthines	Plasma	LiChrosorb RP_8	Water – methanol – triethylamine (pH = 6.0) (43+57+0.25)	UV at 254 nm	0.58-5.76 mg/L	0.02 mg/L	[28]
Flunitrazepam CDZO,Clonazepam, Oxazepam, Lorazepam, Nordiazepam, Diazepam, N-Desalkylflurazepam	Plasma	LC-$_{18}$ DB column (250 mm × 4.6 mm, 5 µm)	5 mM KH2PO4 buffer solution pH6.0:methanol: diethyl ether (55:40:5, v/v/v)	UV at 245 nm	50–1200 ng/mL	30 ng/mL	[29]
CDZO, Clidinium bromide, DicyclomineHCl , Rabeprazole	Capsules	ODS C_{18} RP	Potassium dihydrogen phosphate buffer (0.05 M, pH 4.5 adjusted with 0.5% orthophosphoric acid): Acetonitrile (80:20 v/v)	UV at 215 nm	10-30 µg/mL	0.8603 µg/mL	[30]
CDZO,Other benzodiazepines and their metabolites	Serum	Conventional Reversed-phase C_{18}	Gradient mobile phases: 1.Solvent A (10 mM aqueous ammonium formate containing 0.1% formic acid) 2.Solvent B (methanol containing 0.1% formic acid)	MS/MS, M = 283.2 m/z	5-500 ng/mL	1.0 ng/mL	[31]
CDZO and other benzodiazepines	Oral Fluids	Zorbax Eclipse XDB C_{18}	Solvent A (20 mM ammonium formate pH 8.6; solvent B was acetonitrile. (50:50 v/v)	MS/MS, M = 283 m/z	0.5-40 ng/mL	0.33 ng/mL	[32]
CDZO, other benzodiazepines and their 6 metabolites and zolpidem	Human and rat hair	Zorbax Eclipse XDB-C_{18}	Gradient mobile phase: 1. Solvent A (2 mM ammonium formate/ 0.2% formic acid in water Solvent B (2 mM ammonium formate/ 0.2% formic acid in acetonitrile	MS/MS, M1 = 227.1 m/z M2 = 283.2 m/z	0.5–5 ng/mL	0.05 ng/mL	[33]
CDZO, Other benzodiazepines, flumazenil,zaleplone, zolpidem and zopiclone	Plasma	Merck LiChro CART	Gradient mobile phase: Solvent A (5 mM aqueous ammonium formate adjusted to pH 3 with formic acid Solvent B (acetonitrile)	MS/MS, M1 = 269 m/z M2 = 284 m/z	0.2–3.75 mg/L	0.01 mg/L	[34]
CDZO, other benzodiazepines, opioids, barbiturates, amphetamines and cocaine	Urine	Poroshell EC-C_{18} (2.1 100 mm, 2.7 µm, Agilent)	0.1% acetic acid in water and methanol	MS/MS, M1 = 282.0798 m/z M2 = 227.0499 m/z M3 = 57.0451 m/z	20–500 ng/mL	13.7 ng/mL	[35]

CDZO, other benzodiazepines, Opiates, Synthetic Opiates, and PCP	Waste water	Synergi Hydro-RP	Gradient mobile phase: Solvent A (acetonitrile containing 0.1% formic acid) Solvent B (water with 0.1% formic acid)	MS/MS, M = 227.3 m/z	0.075-10 µg/L	0.075 ng/mL	[36]
CDZO, Other benzodiazepines and hypnotic drugs	Blood	Infinity LabPoroshell 120 EC-C_{18}	Gradient mobile phase: Solvent A (0.05% formic acid in water (v/v) Solvent B (0.05% formic acid in acetonitrile (v/v)	MS/MS, M1 = 227.1 m/z M2 = 283.1 m/z	1–200 ng/mL	0.25 ng/mL	[37]
CDZO and other drugs	Postmortem blood	Raptor Biphenyl columns coupled with Raptor Biphenyl EXP Guard Column Cartridges	Gradient mobile phase: Solvent A (1:1 hexane and ethyl acetate (v/v) Solvent B (dichloromethane/isopropanol/ammonium hydroxide (78:20:2, v/v)	MS/MS, M1 = 227.1 m/z M2 = 283.1 m/z	1–1000 ng/mL	0.4 ng/mL	[38]
CDZO, other benzodiazepines and psychoactive substance	Urine	ACE5 C_{18}	Gradient mobile phase: Solvent A (5%acetonitrile with 0.1% formic acid) Solvent B (95%acetonitrile with 0.1% formic acid)	MS/MS, M1 = 283 m/z M2 = 227 m/z	1-100 ng/mL	0.5 ng/mL	[39]
CDZO, other benzodiazepines, metabolites and benzodiazepine-like substances	Blood	Xterra MS C_{18} column	methanol/formic acid approximately 0.006 M (pH 3, 30–60% (v/v) methanol)	MS/MS, M1 = 284 m/z M2 = 269 m/z M3 = 227 m/z	0.006–2.0 mg/L	1.7 ng/mL	[40]
CDZO, Pharmaceuticals and other abuse drugs	Post-mortem liver	Acquity BEH C_{18}	Gradient mobile phase: Solvent A (water, 0.1% formic acid) Solvent B (methanol, 0.1% formic acid)	MS/MS, M1 = 227 m/z M2 = 282 m/z	5-1500 ng/g	3.51 ng/g	[41]
88 psychoactive drugs include CDZO and their metabolites	Postmortem blood	Hypersil Gold aQ column	Gradient mobile phase : Solvent A (0.1% formic acid) Solvent B (methanol)	MS/MS, M1 = 227.1 m/z M2 = 192.1 m/z	0.05-1 µg/mL	3.2 ng/mL	[42]

2.2 HPTLC methods

Drugs	Matrix	Stationary phase	Mobile phase	Detector	Linearity range	LOD	Ref.
Imipramine HCl and CDZO	Tablets	Precoated silica gel 60 F254 plates	Carbon tetrachloride–acetone–triethylamine (pH 8.3; 6 + 3 + 0.3, v/v/v)	UV at 240 nm	20–240 ng/spot	3.3 ng/spot	[43]

Mebeverine hydrochloride and CDZO	Tablets	Pre-coated silica gel 60F254	Ethyl acetate: methanol (8:4, v:v)	UV at 222 nm	1-14 µg/band	-------	[44]
Mebeverine hydrochloride and CDZO	Tablets	Silica gel plates	Chloroform: methanol: ammonia (9.5: 0.5: 0.1, (v/v/v)	UV at 220 nm	50-600 ng/spot	0.44596	[45]
CDZO and Clidinium Bromide	Tablets	Silica gel 60 GF2S4 plates	Methanol: acetonitrile: water: glacial acetic acid in the ratio 2.0: 6.5: 1.0 :0.5	UV at 217 nm	2.2-12.2 g	-------	[46]

3. Electrochemical methods

Drugs	Matrix	Electrode	Linearity range	LOD	Ref.
CDZO	Tablets	Graphene–carbon paste electrode (GCPE) modified with imprinted polymer nanoparticles (nano-MIP)	6.0×10^{-10}- 7.5×10^{-8} M	2.61×10^{-10} M	[47]
CDZO	Tablets	Mercury electrode & glassy carbon electrode	$2 \times 10^{-7} - 5 \times 10^{-6}$ M	5×10^{-8} M	[48]
CDZO	Tablets & Human serum	Mercury electrode	5×10^{-9} M - 2×10^{-7} M	6.6×10^{-10} M	[49]
CDZO & diazepam	Tablets	Sonogel-Carbon electrode (SngCE) modified with bentonite (BENT)	0.034 – 0.302 µg/mL	16.0 and 5.0 ng/mL	[50]
CDZO	Artificial serum	Hanging mercury drop electrode	8.0×10^{-9} - 9.5×10^{-8} M	$6.6 \times l0^{-9}$ M	[51]
CDZO	Tablets	ion-selective electrodes (ISEs)	-------	6.7×10^{-6} M (CWE) 7.2×10^{-6} M (CWE-T)	[52]

Conclusion

This literature review represents an up to date survey about all reported methods that have been developed for determination of Chlordiazepoxide in its pure form, combined form with other drugs, combined form with degradation products, and in biological samples such as liquid chromatography, spectrophotometry, spectroflourimetry, electrochemistry, etc...

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