

A BRIEF REVIEW ON ANALYTICAL METHODS FOR ESTIMATION OF ISRADIPINE IN PHARMACEUTICAL FORMULATION AND BIOLOGICAL MATRICES

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ABSTRACT

Isradipine (ISRA) is a calcium channel blocking agent and a drug of the dihydropyridine (DHP) class. It is utilized for the treatment of hypertension and congestive cardiac failure (CHF). It selectively binds with the calcium channel receptor with high compatibility and averts calcium alteration into cardiac and arterial smooth muscle cells. ISRA employed to cure deliberate hypertension, used alone or in combination with thiazide diuretics. The present review article represents the compilation and discussion of analytical methods published in the literature for estimation of ISRA in pharmaceutical samples and biological matrices consisting of TLC/ densitometry, HPLC, UV-Visible method, Spectrofluorometry, Capillary Electrophoresis (CE), Polarography, Voltammetry and hyphenated techniques such as LC-MS; LC-MS-MS, GC-MS, etc.

The anticipated review details about the comparative utilization of various analytical techniques for estimation of ISRA. The present compilation can be essentially explored to potentiate future analytical investigation needs of ISRA.

Keywords: Isradipine; hypertension; analytical methods; biological samples; pharmaceutical formulation.

INTRODUCTION

Hypertension is one the most important causes of cardiovascular (CV) morbidity and mortality throughout the world, but in the case, there is widespread agreement that, the treatment of high blood pressure (B.P), over the last half-century has been a huge accomplishment [1]. Hypertension is the world's important risk factor for global disease that is responsible for more than half of the; approximate 17 million deaths per year resulting from cardiovascular disease (CVD) worldwide [2]. Calcium channel blockers (CCBs) were first discovered in the 1960s for the management of hypertension (HT), angina pectoris (AP) and other CVD [3]. Calcium ions play a pivotal role in cellular processes throughout the body, and which also important in the normal function of many types of cells depends on the safeguarding of calcium ion concentration gradient transversely across cell membranes, with the extracellular calcium concentration being about 10,000 times longer than the intracellular concentration [4].

EXPERIMENTAL

Physicochemical properties

Isradipine (ISRA) (Fig.1), is chemically, 3-O-methyl 5-O-propan-2-yl 4-(2,1,3-benzoxadiazol-4-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate. ISRA, differentiate itself from others as DHP derivatives by its benzoxadiazolyl moiety, which contributes to the high affinity for the DHP binding site. The molecular weight and molecular formula of ISRA is 371.393 g/mol and C₁₉H₂₁N₃O₅, respectively. It's practically insoluble in water, having solubility less than 10 mg/L. The melting point for the ISRA is in the range of 168-170°C [5]. ISRA is extremely photosensitive drug and its degradation product don't have therapeutic efficacy [6].

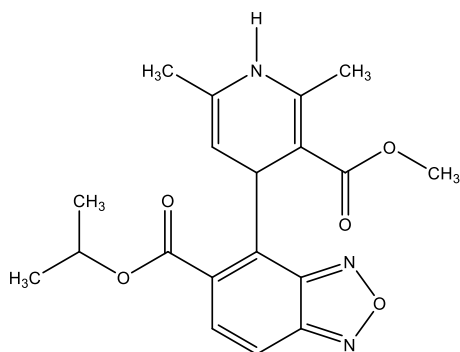


Figure 1. Chemical structure of Isradipine

Mechanism of Action

ISRA acts on smooth muscles which are depolarized primarily by inward calcium ion movement through the voltage-sensitive channel. These calcium ions trigger the release of more calcium from intracellular stores and together bring about excitation-contraction coupling through phosphorylation of myosin light chain kinase (MLCK). The CCBs cause relaxation by the falling intracellular accessibility of calcium ions. They noticeably relax arterioles, but have a mild effect on veins. Extravascular smooth muscle (Bronchial, Biliary Vesical, Intestinal and Uterine) is also relaxed. The DHPs have the most noticeable smooth muscle relaxant and vasodilator act; verapamil is somewhat weaker followed by diltiazem. ISRA, NTP and few other DHPs have been revealed to release nitric oxide from endothelium and inhibit cyclic adenosine monophosphate (cAMP) phosphodiesterase resulting in raising smooth muscle cAMP [7].

Pharmacokinetic and Pharmacodynamic (PK and PD)

ISRA is quickly and almost fully absorbed from the gastrointestinal tract. It depicts broad first pass hepatic digestion bringing about a bioavailability of around 17.00% with oral portions of 0.5 and 0.20 g. The pharmacokinetics (PK) of ISRA are directly in this portion range and peak plasma levels 2-10 µg/ml with case measurement frames achieved inside 2.5 hr. A pharmacodynamic (PD) study concluded that a single dose of orally or intravenously administered ISRA produces arterial vasodilatation as evidenced by the decrease in B.P and systemic resistance in the absence of changes in cardiac filling pressures [8].

Adverse reaction and Therapeutic effects

Adverse drug reaction of ISRA are headache, flushing, ankle oedema, dizziness and tachycardia, that are connected to the drug's vasodilatory action and are commonly encountered with other CCBs. ISRA is mainly used in case of essential HT, stable AP, congestive cardiac failure (CCF) and prevention of atherosclerosis [8].

The particulars of pharmaceutical dosage forms; a route of administration and suggested dose for ISRA is summarized in Table 1. The literature depicts various analytical approaches for determination of ISRA in pharmaceutical formulations, bulk and biological matrices, including LC-MS detection, TLC, HPLC, spectrofluorometric and electrochemical methods. The ISRA was discovered in 1984, which was used in case coronary artery dilator (CAD) and AP. The ISRA is the drug first choice for CAD [9]. The ISRA is mentioned in British pharmacopeia (BP 2005), in which analytical method described for the analysis of ISRA. The ISRA is also mentioned in the United States Pharmacopeia (USP 27, 2004), in which analytical method described for the analysis of ISRA.

Table 1. Pharmaceutical formulation and recommended dose of Isradipine

Pharmaceutical dosage form	Route of administration	Dose
Immediate-release capsules	Oral	Initial dose- 2.5 mg/twice a day The maximum recommended daily dose for the immediate-release capsules is 20 mg
Controlled-release tablets		Initial dose-5 mg orally once a day The maximum suggested dose for the controlled-release tablets is 20 mg/day.

The main objective of the present work is to describe the analytical methods for estimation of ISRA in various formulations and matrices. The compiled data may be explored for the studies on analysis of ISRA. The prominent analytical methods includes Ultraviolet-Visible spectrophotometric and Spectrofluorimetric method (UV-Visible Method), High performance liquid chromatography (HPLC), Gas chromatography (GC), Hyphenated technique like Gas chromatography-Liquid chromatography (GC-MS) and Liquid chromatography-Mass spectrometry (LC-MS) and miscellaneous methods.

Pharmaceutical methods of ISRA

Pharmacopoeial Methods

ISRA is the official drug in the British Pharmacopoeia (BP), United States Pharmacopoeia (USP). BP and USP have reported a HPLC procedure, BP describe the analysis of ISRA using reversed-phase column ODS (Octadecylsillane) (0.10mm x 4.6mm x 5µm) through the mobile phase parts consisting of water: tetrahydrofuran:acetonitrile(625:270:125 v/v/v), at flow rate of 1.2 ml/min and the detection of ISRA at a wavelength of 230 nm [10]. While, in the USP, HPLC analysis was done using reversed phase column(10cm x 4.6 mm), using a mixture of mobile phase composed of water: methanol: tetrahydrofuran (50:40:10 v/v/v), the flow rate at 1.7 ml/min and detection at wavelength at 230 nm [11]. Martindale included the information related to the adverse effects, treatment and precaution [12].

ANALYTICAL METHODS

UV/Visible-Spectrophotometric and Spectrofluorimetric methods (UV-Visible method)

Up till now, only a few UV-Spectrophotometric methods have been reported for ISRA Al-Ghannam et al. executed the reductive analysis of ISRA and Nicardipine (NIC) with Zinc powder and Calcium Chloride and ensured by diminishing with Sodium Pentacyanoaminoferrate (II) to give violet and red

colour products and the analysis was performed at 546 and 539 nm for ISRA and NIC, respectively. The calibration curve was linear over the concentration range of 8.0-180µg/ml and 8.0-110 µg/ml for ISRA and NIC, respectively[13]. Katariya and Prajapati performed the analysis of ISRA from solid lipid Nanoparticles (SLN). Researchers have used methanol and chloroform (7:3 v/v) mixture for dissolving ISRA and detection was performed at 327 nm. The overall % recovery study was found to be 100.80%, which reflects the specificity and selectivity of the method. The authors also described that benchtop stability (up to 1 day) of standard and test preparation, keeping the preparation at a refrigerating temperature; the preparations were reported to be stable [14].

Rao et al. reported a sensitive method for estimation of lercanidipine (LCD) and ISRA in bulk and pharmaceutical formulation. The % recovery of the proposed study was found 99.6 and 99.2 for LCD and ISRA [15].

Al-Ghannam and Al-Olyan demonstrated a spectrofluorometric method for determination of ISRA, NIC and NFD (Nefedipine) in a pharmaceutical matrix as well as in biological samples. Researcher carried out the reduction of ISRA, NIC and NFD with Zn/HCl and measuring the fluorescence intensity achieved ($\lambda_{em}/\lambda_{ex}$) at 460/364, 450/393 and 446/360 nm, respectively. The researcher had also calculated different factors affecting the development of the fluorophore and its stability. Besides this the outcome of surfactant such as β -cyclodextrin (β CD), carboxymethylcellulose (CMC), sodium dodecyl sulphate (SDS) and triton X-100, on the fluorescence intensity were studied and characterized; further, the detection limits of ISRA, NIC and NFD was found to be 0.0028, 0.017 and 0.016 µg, respectively. Hence, the planned study was successfully applied to commercial tablets containing the compounds was found to be 98-101 %. Furthermore, the probable method was applied to urine analysis exclusive of prior extraction demonstrating that there was no interference from the endogenous component and thus revealing high specificity of the planned method [16]. Spectrophotometric and spectrofluorimetric methods for estimation of ISRA are shown in **Table 2**.

Table 2. UV/Visible Spectrophotometric methods for Determination of Isradipine

Sr. No	Drugs	Pharmaceutical Matrix	Wavelength (nm)	Linearity (µg/ml)	Assay (%)	Accuracy study (%)	Ref.
UV/Visible Spectrophotometric Methods							
1	ISRA and NIC	Bulk material	539 and 546	8.0-110 and 8.0-180	101.40 and 100.91	97.64-100.96, 97.64-100.96	13
		Tablet	539	16-80	100.08	98.98- 100.08	
		Capsule	546	32-80	100.35	98.80-100.35	
2	ISRA	Solid Lipid Nanoparticles	327	5 – 30	102.04	99.24- 101.32	14
3	LCD and ISRA	Tablets	236 and 327	2-10	99.6	-	15
Spectrofluorimetry methods							
4	ISRA, NIC and NFD	Tablets	460/364	0.4-6.0		98.5-103.5	16
		Plasma	450/393	0.2-4.0		99.3-101.3	
		Urine	446/360	0.1-9.0		95.3-102.9	

High-Performance Liquid-Chromatography (HPLC)

HPLC an instrumental analytical technique has the capacity to resolve, identifies, and quantifies constituents that are present in a mixture [17]. HPLC is the sort of column chromatography that pumps a sample solution in a mobile phase at elevated pressure through a column containing stationary phase. Because of its capability to sense sample in a trace concentration as low as parts per trillion; HPLC has used a sequence of industrial and scientific applications, such as examination of pharmaceutical substances, environmental samples, forensic study, and chemicals [18,19]. Several researchers have contributed to the establishment of the HPLC method for determination of ISRA from bulk material, pharmaceutical dosage forms and biological samples.

In this review article, we have tried to summarize the different chromatography conditions employed for establishing HPLC methods and its outcomes as well as a conclusion. Among the entire HPLC methods studied, it was found that C18 column was exclusively used for separation of ISRA; since C18 column has the highest degree of hydrophobicity Sorbent phase to retain the ligand molecules [20].

Chaitanya et al. examined ISRA in the pharmaceutical dosage form. Separation of ISRA was accomplished on a kromasil column by using methanol, water and tetrahydrofuran as a solvent system with flow rate kept at 1.7 ml/min. Further, UV (Ultraviolet) detection of ISRA was monitored at 326 nm. The fraction assessment of the ISRA in the marketed preparation reported to be in the range

of 99.61 - 99.86 %, while, limit of detection (LOD) and limit of quantification (LOQ) were mentioned as 0.003 µg/ml and 0.01 µg/ml, respectively [21]. On the same line, Aswini et al. studied HPLC method for estimation on ISRA in a pharmaceutical formulation. In this study separation of ISRA also achieved on a kromasil column at a flow rate 1.4 ml/min using mobile phase composed of methanol, water and tetrahydrofuran. The UV detection of ISRA was monitored at a wavelength of 330 nm [22]. Younus et al. reported HPLC method to estimate the ISRA from tablet formulations. The linear relation between the peak area and concentration was recognized with a coefficient of correlation value of 0.9998. The developed method was set up precisely with intra-day and inter-day mean recovery was found to be 99.89 and 99.65%, respectively [23]. Pasumarthy et al. reported HPLC method to quantify ISRA from capsule formulation. The separation of ISRA was achieved using methanol, acetonitrile and acetate buffer (pH 2.8) with flow rate at 1 ml/min. The developed strategy was successfully applied for estimation of ISRA in capsule formulation and % mean recovery was found to be 99.66 ± 1.20 % this demonstrating high accuracy and precision [24]. Rao et al. reported the executed analysis of ISRA in the capsule dosage form using reverse phase column using methanol and water as the mobile phase with a flow rate of 1 ml/min. The calibration curve for the planned method was linear above the 1-100 µg/ml with (r^2) = 0.999. The proposed analysis was mainly carried out to quantify ISRA in the capsule dosage form. For determination of ISRA 5 mg of the drug was analyzed and average drug content was found to be 97.80 % [25]. Simoes et al. created enantioselective liquid chromatographic technique using two-phase hollow fibre liquid-phase micro extraction (HF-LPME) HPLC for the determination of ISRA enantiomers and its metabolite (ISRA is apyridine derivative). The given sample analytes were extracted from 1000 µl of microsomal medium which has a binary-phase HF-LPME system with hexyl acetate as the acceptor segment of that system. Furthermore the developed method effectively useful to an in-vitro biotransformation study of ISRA. [26].

Rafaat et al. depicted cyclic Voltammetry with a glassy carbon electrode and the spectroelectrochemical properties of ISRA. Furthermore, based on this study a HPLC method with electrochemical detection was accomplished to estimate ISRA in serum. For the development of HPLC method, researchers applied electrochemical detection with polybutadiene coated alumina column employing an alkaline mobile phase. The calibration curve of this method was linear above the concentration range of 0.2–200 ng/mL with (r^2) = 0.9924.

The intra and inter-day studies were examined for control human serum and proportion relative standard deviation range from 0.5 to 6.7 [27].

Al-Suwayehet al. reported the a simple and sensitive HPLC method with short, timely analysis and utilized small plasma samples (0.25 ml). A liquid phase extraction method was preferred for extracting out the ISRA sample from plasma and diazepam was used as the internal standard to increase precision and reproducibility. Accurately 90.34 to 99.79% and 84.6 to 107.2%, analysts were recovered from the plasma samples [28].

Bidouil et al. has developed HPLC method for the study of ISRA oxidation in vitro; further proposed established method was applied in vitro metabolism by h3A4/OR cells. The researcher determined the ISRA and its main metabolite after the extraction from the RPMI+9% HS (culture media) and from the h3A4/OR cells. The chromatographic separation was performed using an isocratic system with Chromospher C8 column (10 cm x 0.46 cm I.D., 5 µm particle size), detection of analytes was monitored at 240 nm using the photodiode array detector. The main objective of the proposed work is to measure the cytochrome P450 3A4 isoenzyme activity in h3A4/OR cells [29]. Oravcova et al. performed stereoselective binding of ISRA to human plasma proteins have been studied in vitro over an ISRA concentration (0.06–20 µmol/l) using HPLC [30]. On the same path, Angelo et al. described an enantioselective method for estimation of ISRA from human plasma using HPLC and GC (Gas chromatography). Researchers employed alkaline extraction into hexane for quantitative separation of enantiomers of ISRA using HPLC; analysis was performed on a Chiralcel OJ column at 39°C. Further collected fractions of sample analytes were evaporated and assayed by means of capillary gas chromatography on an HP 50+ column with NPD. The Authors reported that as a result of 2.0 ml of serum, 0.7 nmol/l (0.26 ng/ml) of each enantiomer could be resolved with satisfactory accuracy [31]. Mielcarek et al. reported studies on a photochemical breakdown of ISRA (IS) and its liquid inclusion complexes with methyl-β-cyclodextrin (M-β-CD). The photon energy deprivation of ISRA was executed using UV-Spectrophotometry, HPLC and HPTLC methods. Researchers also reported that the HPLC method is differentiated by greater sensitivity, however, dejectedly additionally a lower precision, than the UV spectrophotometry [32]. Elghany et al. has developed a stability indicating HPLC method for assessment of ISRA in bulk, tablet and in a capsule dosage form. The separation of investigation was achieved within 20 min and detection was performed at 325 nm. The ISRA was subjected to acid, base and hydrogen peroxide degradation and degradation studies revealed that no peak interference between ISRA and degradation products. The proposed method was sensitive to 0.25 µg/ml [33]. The different HPLC methods for determination of ISRA in a pharmaceutical matrix as well as in biological samples were summarized in **Table 3**.

Table 3. HPLC methods for determination of Isradipine

Sr. No	Drugs	Pharmaceutical or Biological matrix	Column	Chromatographic conditions	Mode of Analysis	Linearity (µg/ml) (ng/ml)	References
1	ISRA	Tablet	Kromasil C8 column. (100 × 4.6 mm × 5 µm)	Water: methanol:tetrahydrofuran, (500:400:100 v/v/v), column oven temperature 25°C and flow rate 1.7 ml/min	Isocratic	50-400	21
2	ISRA	Tablet	Kromasil C8 column (100 mm × 4.6 mm × 5 µm)	Water: methanol: tetrahydro furan, (50:40:10 v/v/v), column oven temperature 25°C and flow rate 1.4 ml/min	Isocratic	10-60	22
3	ISRA	Tablet	Agilent Zorbax C8 (150 mm × 4.6 × 5 µm)	Methanol: acetonitrile: 0.1% OPA (55:35:10 v/v/v), column oven temperature 30°C and flow rate 1.0 ml/min	Isocratic	10 to 150	23
4	ISRA	Capsule	C18 column	Methanol: acetonitrile: acetate buffer pH 2.8 (60:30:10 v/v/v), column oven temperature 40°C and flow rate 1.0 ml/min	Isocratic	50-600	24
5	ISRA	Capsule	C18	Methanol: water (70:30 v/v), column oven temperature 40 °C and 1.0 ml/min	Isocratic	1-100	25
6	ISRA	(HF-LPME)	Chiralpak® AD column (250 mm × 4.6 × 10 µm)	Hexane: 2-propanol: ethanol (94:04:02, v/v/v), column oven temperature 23°C and flow rate 1.5 ml/min	Isocratic	50–5,000 and 50–2,500	26
7	ISRA	Bulk form	(Aluspher RP- Select B, 250 × 4 mm × 5 µm I.D)	0.1 M Britton–Robenson Buffer (pH 11.8): acetonitrile: methanol (6:3:1 v/v/v), flow rate 1.0 ml/min	Isocratic	2–200	27
8	ISRA	Bulk form and Human plasma	Column Kromasil C8 5 µm, (150 × 4.6 × 5 µm)	Water and Acetonitrile pH 4.0 (50:50 v/v) and flow rate 1.8 ml/min	Isocratic	10-120	28
9	ISRA	Bulk form and Rabbit plasma	Chromspher C8 Column (100 × 4.6 mm × 5 µm)	55.4% water with 0.16 M NaH ₂ PO ₄ and 0.017 M tetrabutylammonium hydrogensulfate, 43.6 % of methanol and 1% Tetrahydrofuran pH of aqueous solution up to 3 and flow rate 1.8 ml/min	Isocratic	1.0-100	29
10	ISRA	Bulk form and Human Plasma	C18	NaH ₂ PO 0.20 M in water: methanol (55: 45 v/v/v), column oven temperature 30°C and flow rate 1.0 ml/min	Isocratic	(0.06-20 µmol/l)	30
11	ISRA	Rac Mixture of ISRA and Human Plasma	Chiral Column 25 cm × 4.6 mm I.D Chiralcel OJ	9.5% 2-propanol in Hexane, 39 °C and 1 ml /min	Isocratic	-	31
12	ISRA	Human Plasma	LiChrospher 100 RP-18 column (250 mm × 4 mm × 5 µm)	Methanol: water: acetonitrile (7:3:4.2 v/v/v), room temperature and flow rate 0.8 ml/min	Isocratic	7.0 × 10 ⁻⁶ – 14.0 × 10 ⁻⁵ mol IS/ l	32
13	ISRA	Bulk form and Capsule	ODS column (Ultrasphere, 15 cm × 4.6 mm I.D., 5 µm)	Methanol: water (60:40, v/v), column oven temperature 23°C and flow rate 1ml/min	Isocratic	10-60	33

Gas chromatography (GC)

Christensen et al. developed simple and precise GC method for determination of ISRA and its metabolite in serum. Simple one-step extraction procedure, wide-bore and nitrogen phosphorus detector were employed and a limit of quantification of ISRA and its metabolite was found to be 0.5 and 2.0nM, respectively. Further, the proposed method successfully applied to PK study in hypertensive women during pregnancy [34].

Hyphenated techniques

The hyphenated technique is established by the association of chromatography and an on-line spectroscopic technology to explore the improvement of both techniques [35]. The hyphenated technique consists of two major parts of a separation method (GC, HPLC or electrophoresis) and a detector (UV, AAS, ICP-MS or ESI-MS) these are linked by an appropriate interface. The hyphenated techniques, mostly implemented for fast identification and categorization of known or unknown compounds. Further, the hyphenated technique offers the several advantages over other techniques such as better reproducibility, higher sample throughput and little analysis time [36]. Only a few hyphenated methods were developed and validated for the assessment of ISRA in biological fluids and pharmaceutical dosage form.

Gas chromatography-Mass spectrophotometry (GC-MS)

Jean and Laplanche, have developed capillary GC-MS method for the analysis of ISRA and five its metabolite in plasma and urine samples. The separation of analytes were performed on the chromatography column was a 10 m x 0.53 mm I.D. fused-silica open column coated with 2.65 μm of a Cross-linked methylsilicone (C-LMS) gum and methane with flow-rate was used to 10 ml/min. The negative-ion mass spectrometry with chemical ionization (CI) was used for detection [37]. Maurer and Arlt developed and reported a GC-MS is showing procedure for detection of dihydropyridine calcium channel blocker basically calcium antagonist metabolites (AMD, FLD, ISRA, NFD, NVD, NMD, NSD, and NTD) in urine after intake of remedial doses. The extractive methylation was working for the systematic toxicological analysis method for acidic drugs and poison. Further, the division of compounds was separated by capillary GC and characterized by computerized MS in the full scan mode. Researchers reported that the overall % recovery ranged between 67-77 % with a coefficient of variation below 10 %, likewise, the LOD was at most 10 ng/mL [38].

Liquid-Chromatography-Mass spectrophotometry (LC-MS)

Baranda et al. reported distinctive type research of Instability of CCB's through sample preparation for LC-MS-MS analysis of serum samples. In the projected work, the researchers have been testing stability of these compounds in plasma were tested by exposure of plasma samples to the light at room temperature. Further, the products of photodegradation were identified and characterized using LC-UV-DAD and LC-MS-MS, respectively [39].

Bartlett et al. labelled LC-MS method for the determination of degradation products of ISRA. For resolve of its degradation products, ISRA was exposed to different stress conditions like acidic, basic, oxidative, heating and UV light. After exposure of ISRA, it was found that ISRA was highly stable in acidic condition; while in basic condition conversion of the methyl ester to the corresponding carboxylic acid. Exposure to UV light caused 20% of the ISRA was converted from the DHP form to the pyridine analogue within 24 hours and exposure to oxidizing conditions caused broad degradation of the sample. From these, the researchers concluded that the ISRA should be stored in a tightly sealed container and protected from light [40].

Kang et al. reported susceptible and fast LC-MS method for the analysis of ISRA in human plasma using AMD as internal standard. A simple liquid-liquid extraction procedure was employed for the extraction of analytes from the biological matrix using methyl-t-butyl ether after alkaline treatment. The separations of analyte were performed using RP-HPLC and detection was done by positive ion ESI in multiple reaction monitoring (MRM) mode. The proposed method obeyed good calibration curve in the range of 10 to 5000 pg/ml with (r^2) = 0.9998 and the LLOQ were originated to be 10 pg/ml.

Furthermore, the proposed method was effectively applied to the bioequivalence and PK studies of 5 mg of sustained-release ISRA in 24 healthy Korean volunteers [41]. Yuan et al. developed the UPLC-MS/MS method for the analysis of ISRA in dog plasma. The separation of analytes of the samples was achieved using acquity UPLC BEH C18 column with positive ion ESI mode. The 0.2 ml/min total flow rate was kept throughout the analysis and chromatographic run time of each sample was 1.4 min. The calibration curve was linear above the concentration range of 0.1-40 ng/ml. In addition to this, the well-known method showed good sensitivity with a LLOQ 0.10 ng/ml [42]. The hyphenated techniques employed for the estimation of ISRA was shown in **Table 4**.

Table 4. Hyphenated techniques for estimation of Isradipine

Sr. No	Drugs	Pharmaceutical or Biological matrix	Chromatographic conditions	Internal standard	Detector	Mass to charge ratio	Ref.
Gas chromatography-Mass spectrophotometry							
1	ISRA	Plasma and Urine	Column was (10 m x 0.53 mm i.d.), Methane was used as carrier gas with flow-rate was adjusted to 10 ml/min and temperature was 350°C	[¹³ C ₄]-PN 200- 110 and 202-479	Mass detector	311, 315 and 339	37
2	AMD, FDP, ISRA, NFD, NVD, NMD, NSD, and NTD	Urine	Capillary column (12 mm, 0.2-mm i.d.), Helium was used as carrier gas with flow rate 1 ml/min and temperature 100 to 300 °C	-	Mass detector	139, 284, 297, 298, 310, 312, 313, 318, 324, and 332	38
LC-MS/MS							
3	AMD, NCD, LCD, NFD, NTD, ISRA, NMD, NSD and FLD	Bulk material	Luna RP-C18 (150 mm x 2 mm 3.0 μm , i.d.), using 0.1% formic acid in 1 mM ammonium formate, (pH 2.7); (A) and 0.1% formic acid in acetonitrile and 1 mM ammonium formate (B) (95:5, v/v).	D5-diazepam and D3-doxepin	LC-DAD and mass quadrupole	-	39
4	ISRA	Bulk material	Ultrasphere C18 (15 x 4.6 mm, 5 μm , and i.d.), acetonitrile: water; gradient mode of analysis; with flow rate 1 ml/min and column oven temperature- 25°C.	-	Triple mass quadrupole	394	40
5	ISRA	Human plasma	YMC Hydrosphere C18 (50 mm x 2 mm, 3 μm , i.d.), methanol:20 mM ammonium acetate (90:10 v/v) with flow rate 0.25 ml/min.	AMD	Triple mass quadrupole	372.1	41
6	ISRA	Dog plasma	BEH C18 column (50 mm x 2.1 mm, 1.7 μm , i.d.), acetonitrile:water: formic acid, (80:20:0.3, v/v/v) with flow rate 0.2 ml/min.	Methyltestosterone	Triple mass quadrupole	372.4	42

Miscellaneous methods

Belal et al. has proven an anodic polarographic determination of ISRA in pharmaceutical dosage forms. The anodic behaviour of ISRA considered using the cyclic direct current, voltammetry and polarography. ISRA was shown definite anodic polarographic waves over the pH range of 04-08 in Britton-Robinson buffer (BRB). At given pH 06, the analytical pH, the diffusion-current constant (Ilkovic Equation) was $2.12 + 0.42$ ($n = 4$). The oxidation potential was -0.10 V vs Ag/AgCl reference electrode with coefficients of correlation values of 0.9977 and 0.9948 in the direct current and differential pulse polarography respectively. The current-concentration curve of samples plots were rectilinear over the ranges 05-14 and 2.4-12 $\mu\text{g/ml}$ using the direct current and differential modes, respectively and the LOD was 1.12 $\mu\text{g/ml}$ [43].

Belal et al. developed the voltammetric method for estimation of ISRA in a tablet dosage form as well as in biological samples. The current-concentration of sample plots were straight-lined over the range 01-20 and 0.1-18 $\mu\text{g/ml}$ using the direct current and differential pulse modes, respectively, with lowest detectability of 0.01 mg/ml. The fraction recoveries in spiked human plasma and urine were found to be 103.88 ± 5.13 and 100.12 ± 1.42 [44].

Fernando et al. developed a simple enantioselective method using CE for the determination of ISRA in pharmaceutical formulation and estimation of enantiomers in degradation studies. The separation of enantiomers was achieved using Sulfobutylether β -cyclodextrin (SBE-b-CD) as a chiral selector and a simple sodium borate buffer. The calibration curve was linear over the concentration range of 50-300 $\mu\text{g/ml}$ for both enantiomers with a coefficient of correlation value of 0.9978. Besides, the degradation results showed that the established method was selective and stability indicating and ISRA enantiomers were successfully separated from the oxidative degradation product and formulation excipients [45].

CONCLUSION

The present review described the summary of all analytical methods which are reported in the literature for the estimation of Isradipine not only in bulk, pharmaceutical formulations but also in biological matrices. Isradipine is 1,4-DHP derivative, particularly employed as antihypertensive agent; apart from lowering blood pressure; it may lead to another beneficial effect for instance stable AP, CHF and prevention of atherosclerosis. Analytical methods like LC-MS, GC-MS, UPLC, HPLC, HPTLC, UV/Visible Spectrophotometer, spectrofluorometry, CE, Voltammetry and polarography were employed for quality control determination of ISRA in pharmaceutical dosage forms and biological matrix.

The primary objective of the compilation of review is to collect maximum information available on analytical methods of Isradipine and study it in detail. From this survey, it is revealed that very few analytical methods are obtainable on HPTLC, UV/Visible spectrophotometry, electrochemical methods.

The reported data for analysis of isradipine revealed that HPLC is the most frequent technique was employed for the determination of isradipine in pharmaceutical matrix. For analysis of isradipine in pharmaceuticals HPLC with UV detection is an appropriate due to this strategy gives precise outcomes and minimal effort contrasted. By employing MS techniques to LC offered unique selectivity and sensitivity as well as a choice of method for analysis of isradipine and its metabolites in biological samples. Hyphenated techniques such as GC-MS and LC-MS, LC-MS/MS and UPLC-MS/MS methods are also reported for quantification of isradipine in plasma and other biological fluids.

The analytical methods implemented in the studies on Isradipine are shown in Table 2, 3 and 4. Its likely to overview the dissimilarities between reported methods. Up till today there were 27 analytical methods found in which validated methods and its application in the determination of Isradipine in pharmaceutical dosage forms and biological matrix. Of those, three methods were developed by using UV-Visible method, a single Spectrofluorometric method, thirteen (HPLC) methods, a single Gas chromatography (GC) method, six Hyphenated methods (LC-MS, GC-MS) and three miscellaneous methods. (Polarography, Voltammetry and CE).

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