DOI: 10.15415/jptrm.2017.51004

Analytical Review on Raloxifene -An Estrogen Receptor Modulator in Different Pharmaceutical Formulations and Biological Fluids

ASHWINI V. GUJAR, ANAND B. MUNDADA AND ATUL A. SHIRKHEDKAR*

Department of Pharmaceutical Chemistry, R. C. Patel Institute of Pharmaceutical education and Research, Shirpur, Dist. Dhule - 425405, India

***Email: shirkhedkar@gmail.com**

Received: Dec. 15, 2016 | Revised: Feb. 20, 2017 | Accepted: March 22, 2017

Published online: May 05, 2017

Abstract Raloxifene (RLX) is an oral selective estrogen receptor modulator (SERM). It is showing estrogenic action on bone and anti-estrogenic action on uterus and breast. An extensive literature has been published for analysis of RLX in different pharmaceutical formulations. This review article endeavor to provide the detail account on analytical methods for RLX and also validation details for its readers. It further helps to avoid costly chemicals and time consuming exercises for further investigation of RLX.

Keywords: Analytical Method; Raloxifene; RP-HPLC; HPTLC; Validation

1. INTRODUCTION

Raloxifene (RLX) is an oral selective estrogen receptor modulator (SERM). It is showing estrogenic action on bone and anti-estrongenic action on uterus and breast.RLX is chemically[6- hydroxy-2-(4-hydroxyphenyl)benzo[b]thien-3-yl] -[4-[2-(1-piperidinyl) ethoxy] phenyl] Methanone (**Figure 1**). It belongs to benzothiophene derivative. RLX is used in the treatment and prevention of osteoporosis in post-menopausal women by reducing resorption of bone and increasing bone mineral density. [1] This effect is produce by ERα & ERβ receptor where it acts as partial agonist. RLX is preferred drug in the treatment of breast cancer because of its antagonist action at this site. [2]

Analytical chemistry deals with the techniques for separation, identification and also for quantification of chemical component such as natural and artificial origin. [3] The preference of any analytical technique is

Journal of Pharmaceutical Technology, Research and Management
Volume 5, No. 1,
May 2017
pp. 41–57

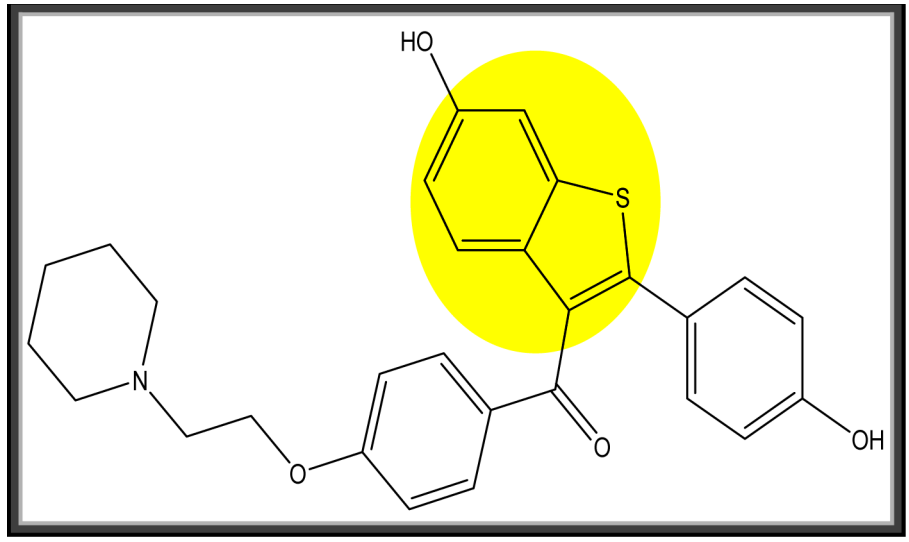

Figure1: 2D Structure of RLX.

based on much contemplation which includes chemical properties of analyte, its concentration, cost of analysis and also on the type of estimation either qualitative or quantitative.

A qualitative technique gives knowledge about the chemical identity of the species in the sample. A quantitative technique gives numerical data about one or more analyte in the matrix sample.The most explored techniques for quantitative analysis of drugs and its metabolite in blood sample includes High-Performance Liquid-Chromatography (HPLC), Thin–Layer Chromatography (TLC) and also hyphenated techniques such as LC-MS, LC-MS-MS, LC-NMR etc [4, 5]

The review article attempts to compile the date of methods applied for analysis of RLX in bulk, pharmaceutical formulation and biological matrix. Further the discussion about some prominently applied analytical methods is also included.

2. ANALYTICAL ESTIMATION OF RLX

The analysis of drugs is an inclusive part of drug development process.RLX is official in Indian pharmacopoeia and suggested the RP-HPLC method by applying stainless steel column 150 × 4.6 cm packed with octylsilane bonded to porous silica (3.5 μm) and acetonitrile and phosphate buffer (33:67 *v/v*) pH of the resulting solution was maintain at 2.5 which was adjusted by aqueous solution of potassium hydroxide or orthophosphoric acid. The RLX was detected done by spectrophotometer at 280 nm.[6]

2.1 Spectrophotometry/ Spectroscopy Methods for Analysis of RLX

In most of these UV- Spectrophotometricmethods, Methanol and NaOH (0.1N)were used as a solvent. In methanol and NaOH (0.1 N) solvents RLX exhibitabsorbance maximumat 289 nm and 303 nm, respectively**.** The bathochromic shift in wavelength of absorbance maximum for RLX in 0.1NaOH may be due to solvent effect. The valued information about these methods viz solvent used, detection wavelength linearity and range, coefficient correlation value has been furnished in **Table 1.** [7–15]

2.2 Colorimetric Methods

Four Colourimetric methods have been studied for analysis of RLX. A colour chromogen of RLX was formed by reacting it with different reagents. The

Table1: UV- Spectrophotometric Method for Determination of RLX.

Sr. No.	Sample	Methods	Detection Wavelength (nm)	Linearity Range (μg/ml)	r^2 Value	Solvent	Reference
1	Tablet	Zero Order	720	8 – 24	0.999	0.1M Sodium Hydroxide	7
2	Tablet	Zero Order	555	16 – 48	0.999	Methanol: Water mixture	8
3	Tablet or Bulk	Zero Order AUC First Order	303.5 275 – 350 303-385	2 – 10	0.999	0.1N Sodium Hydroxide	9
4	Tablet	Zero Order	288	2–12	0.999	Methanol	10
5	Tablet	Zero Order	665 555	0.5 – 5.0 – 2.0	0.9997	Distilled water	11
6	Tablet	Zero Order	649.3	10 – 30	0.9998	Methanol	12
7	Tablet	Zero Order	520 610	0.1–2.0 0.5 – 6.0	0.9993 0.9989	Water	13
8	Tablet	Zero Order	624.4	16 – 48	0.9999	Methanol	14
9	Tablet	Zero Order	284	3.0–12.0	0.9994	Methanol	15

intensity of colour was recorded at the appropriate wavelength. The details about the methods are given in **Table 2.**

Table 2: Colorimetric Method used for Determination of RLX in tablets.

Sr. No.	Solvent (s)	Reagents	Detection Wavelength (nm)	Linearity Range (µg/ml)	r^2 Value	Reference
1	Methanol	Method A Aqueous solution of sodium hydroxide (0.1M)	425	5– 150	0.999	17
		Method B Ferric chloride (0.03M) and 1, 10-phenanthroline (0.01M)	510	1 – 10		
		Method C Ferric Chloride (0.03M) and 2,2'bipyridyl (0.01M).	521	2–25		
2	Methanol	Tetracyanoquinodi ethane(TCNQ)	842.5	2–16	0.999	18
		FolinCiocalteu's reagents	750	4–32	0.997	
3	Methanol and Distilled water	Method A Methanol as a solvent	289	5–25	0.9993	19
		Method B 0.1 M Sodium Hydroxide	303	5–25	0.9997	
		Method C Ferric nitrate and 1,10-phenanthroline	511	2–10	0.9987	
4	Methanol	Method A permanganate in acetic acid medium	430	0.6–6.0	0.999	20
		Method B of permanganate in H_2SO_4 medium	550	1.5–15.0	0.998	

B. Kalyanaramu et al. established a simple and easy method for quantification of RLX in bulk and pharmaceutical preparations by visible spectrophotometry. Analytical method illustrated for RLX was suggested to be depend on creation of dark green colored chromogen complex with sodium nitroprusside in presence of hydroxyl amine under alkaline conditions. The detection of RLX was performed at wavelength 720 nm. The developed method was implemented for analysis of RLX in pharmaceutical formulation. The author depicted the mechanism for the formation of the colored chromogen. RLX is acting as a donor because of availability of cyclic tertiary nitrogen in piperidine portion and sodium nitroprusside in occurrence of hydroxylamine and alkali exists as aquoferrocyanide [Fe (CN) $5H_2O]^{3-}$.The mechanism of formation of colour chromogen is depicted in **Figure 2**. [16]

2.3 Differential Spectrophotometry Method

One method was reported using differential Spectrophotometry technique. Two different solutions of RLX were prepared using HCl(0.1 N)and NaOH (0.1N). These solutions further diluted with the respective solvents and scanned in the UV- range. RLX in HCl (0.1 N)exhibited maximum absorbance at 289 nm whereas in NaOH (0.1 N) it exhibited absorbance at 333.4 nm. The difference absorption spectrum (ΔA) of alkaline RLX was measured using basic solution in sample cell and acidic solution in reference cell.[21]

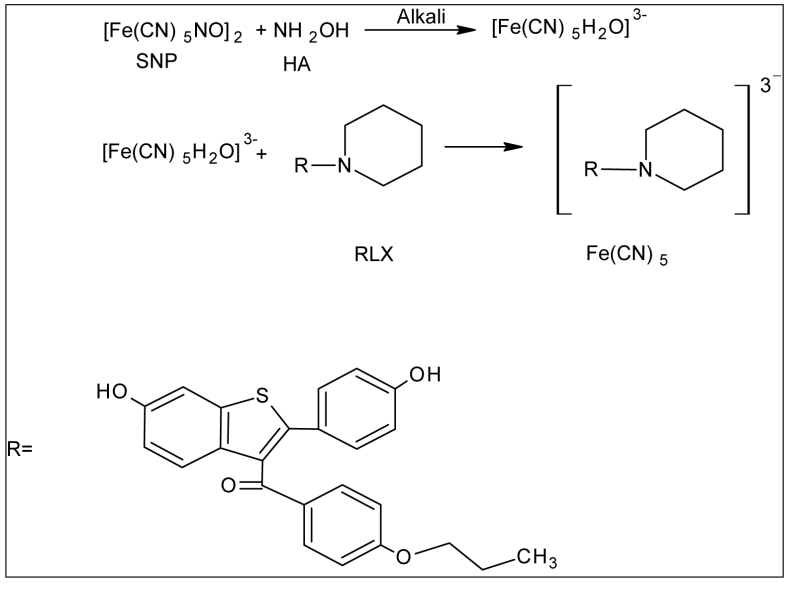

Figure 2: Mechanism of formation of colour chromogen complex of RLX.

2.4 Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC)

Currently RP-HPLC is the most explored analytical tool for separating complicated mixture of chemical, pharmaceutical and biotechnology industry. RP-HPLC is the opposite to normalphasechromatography. In RP-HPLC the stationary phase is nonpolar and mobile phase is polar. The most common stationary phases used is octadecyldimethyl (C18) with silicaas the solid support. Silica has a narrow pH range (3–8) where mixtures of component can be separated without deteriorating the column performance. Above pH 8, these columns are not suitable due to dissolving of silica and it may damage the column. Below pH 3, the silicon-carbon bond is broken and the column be damaged.Theseparation is attend byanalytes having different interactions with the stationary phase. In RP-HPLC,solutes are resolved on the basis of their hydrophobicity. A highly hydrophobic solute will beretained on the column for longer period of time than a less hydrophobic one. Further, polar solutes will interact withthe silica surface to cause peak tailing. The mobile phase is one of the two components involvedin the resolution. Water is generally one of the components of a binary mixture in RP-HPLC.Water is considered to be the weak portion of the mobile phase and does not interactwith the hydrophobic stationary phase.[22– 33]

Thirteen RP-HPLC methods have been studied for analysis of RLX in pharmaceutical matrix . In many of these methods UV- visible detector and Diode array detector has been implemented. Overall ten methods have implanted isocratic mode for analysis of RLX and only three methods reported gradient mode of analysis. The details about the mobile phase composition columns, detector, Detection and linearity, validation parameters etc. are summarized in **Table3.**

2.5. Determination of RLX and its Impurities

Impurity profiling is of great significance in novel drug substance and new drug product due to their potential undesirable pharmacological effects., possible toxicity, adverse effects. Ultimately it has large impact on the activity, efficacy and the stability of drug substance and its bioavailability. So there is enduring requirement for establishing new simple accurate, specific methods for estimation of drug impurities. There are various sources of impurities; they are from starting material; intermediates and by-product from the synthesis of active pharmaceutical ingredient(Process related impurity); degradation product emerged during manufacturing process and also through storage of product for longer period; and also interaction product between API and other active ingredients and excipients or primary container . [34-36] Very few research papers are reported on impurity profiling of RLX.

Sr. No.	Mobile phase composition	Mode of analysis	Column type and make	Column specification	Column temperature	Flow rate	Detector	Detection wavelength	Retention time	Linearity	R2 value	Reference
1.	Acetonitrile :Water (30:70)	Gradient	Kromasil C18	150x4.6mm 5 μm.	RT	1ml/ min	UV/Vis detector	280	1.00	-	0.999	22
2.	Acetonitrile : Phosphate buffer (30: 70 v/v)	Gradient	RP C18	150x4.6mm 5 μm.	RT	1mL/ min	UV dual absorbance detector	290	10.609	0.5- 200	0.9997	23
3.	Acetonirile and Ammonium acetate buffer in the ratio 75:25v/)	Gradient	Inertsil, ODS C18	250x4.6mm 5 μm.	Ambient	1mL/ min	PDA detector	254	4.4	2.5 – 12.5	0.999	24
4.	Buffer :Acetonitrile (60:40 % v/v)	Isocratic	C18 XTerra	150x4.6mm 5 μm.	RT	0.8mL/ min	UV/Vis detector	287	4.890	10 - 50	0.9999	25
5.	Buffer and acetonitrile (64:36% v/v)	Isocratic	ZodiacSil C4	150x4.6mm 5µm	25°C	0.7mL/ min	PDA detector	287	4.011	2.5-15	0.999	26
6.	Water (the pH was adjusted to 3.9 with glacial acetic acid), acetonitrile and triethylamine (65.75:34:0.25: v/v/v)	Isocratic	C18	250X4.mm, 5µm	RT	1mL/ min	UV/Vis detector	287	5.51	0.5-50	0.999	27
7.	64% 50mM phosphate buffer with pH adjusted to 3.0 with o-phosphoric acid and 36% acetonitrile	Isocratic	Nucleosil C18 5	250X4.0mm 5 µm	37°C	0.7mL/ min	UV and Colorimetric detector	287		0.5-10	0.999	28

Sr. No.	Mobile phase composition	Mode of analysis	Column type and make	Column specification	Column temperature	Flow rate	Detector	Detection wavelength	Retention time	Linearity	R2 value	Reference
8.	Acetonitrile: Methanol :Aqueous Ammonium acetate buffer (60:10:30 v/v/v).	Isocratic	Kromasil-100, C-18	250 x 4.0 mm, 5 μm	40°C	1ml/ min	PDA detector	284	2.480	0.05-250	0.9999	29
9.	Mobile phase A (0.154% Ammonium acetate solution, pH adjusted to 4.0 with acetic acid) : Mobile phase B (Acetonitrile)	Isocratic	Hypersil ODS column	250 X 4.6 mm 5.0 µm	40°C	1mL/ min	PDA detector	284	6.65	50 – 600	0.9991	30
10.	Phosphate Buffer (pH-6.8) : Acetonitrile (60:40v/v)	Gradient	RP C18	100×4.6mm 3 μm.	Ambient	1mL/ min	UV detector	287	3.814	20-120	0.993	31
11.	Water:Acetonitrile: Triethylamine pH-3.5(adjusted with o-phosphoric acid) (67:33:0.3 v/v)	Gradient	NST C18	250×4.6mm 5 μm.	30°C	1mL/ min	UV detector	287	7.64	4.0-16.0	0.999	32
12.	methanol and water (50:50 v/v)	Gradient	Water's Symmetry C_{18}	4.6×150 mm .	RT	1mL/ min	2487 dual absorbance detector	230	4.1	10-60-	0.999	33

European pharmacopoeia reported three impurities mainly along with their chemical structuresincludes[6-Hyroxy-2-(4-hydorxyphenyl)-7-[4-[2-(piperidin-1-yl)ethoxy]benzoyl]-1-benzothiophen-3-yl][4-[2-(piperidin-1-yl)ethoxy]phenyl]methanone (impurity 1);[6-Hyroxy-2-(4-hydorxyphenyl)-1-benzothiophen-7-yl][4-[2-(piperidin-1-yl) ethoxyl]phenylmethanone(impurity2);[6-Hyroxy-2-(4-hydorxyphenyl)-1-benzothiophen-3-yl][4-[-2((piperidin-1--yl)ethoxyl] phenyl]methanoneN-oxide(impurity 3)[37]. The impurity 1 is also reported in United States Pharmacopeia.[38]

Improvement in optimization of separation of impurities is achieved by recently established chromatographic techniques, applications of it liquid chromatography- separation of RLXand itsimpurities have beenstudied. Research paper explored about the chromatographic separation of RLX and its impurities using analytical column was x Bridge™ 100 mm x 3 mm, 3.5 μm particle size columns. Acetonitrile: Water (5 mM-SDS, pH 2.8) mixture of 45:55% v/v was selected as a mobile phase and detection of drug and impurities were performed at 254 nm with flow rate 1mL / min.

The central composite design experimental plan using new chromatographic response function (CRF) was the major outcome of the system. [39]

N. Jagdeesh et al reported structural interpretation of the most potent impurities of RLX by LC/ESI-MS and NMR. Three impurities were detected during the impurity profile studies of RLX by reversed phase gradient HPLC method. These three impurities displayed identical molecular ion at m/z488. [40]

R.B. Reddy et al established detection and characterization of potent impurities in RLX. At the time of synthesis of the bulk drug RLX, eight impurities were reported. Out of which four were reported to be new. All these impurities identified using gradient high performance liquid chromatography method. LC-MS was studied to detect the mass number of the these impurities and systematic protocol was followed to characterize them. These impurities were also synthesized and characterized. Further the confirmation has been done by co- injecting the synthesized impurities with the RLX in HPLC. The confirmation has been done on the basis of matching the spectral and chromatographic data. [41]

Out of eight reported impurities, three are already reported in European pharmacopoeia. [37] The chemical name of remaining impurities [6,6'-Dihydroxy-2,2'-bis(4-hydroxyphenyl)-7,7'-bi-1-benzothiophene-3,3'-diyl]bis{[4-(2-piperdin-1-ylethoxy)phenyl]methanone}(impurity4); 6-Acetoxy-2-[4-hydroxyphenyl]-0-benzothiophene (impurity 5); Methyl 4-[2-(piperidin-1-yl)ethoxy]benzoate (impurity 6); 1-[6-hydroxy-2-(4-

hydroxyphenyl)-1-benzothiophen-3-yl]ethanone (impurity 7); 7-Acetyl-[6 hydroxy-2-(4-hydroxyphenyl)-1-benzothiophen-3-yl][4-[2-(piperidin-1-yl) ethoxy]phenyl methanone (impurity 8)The chemical structures of impurities 1- 8 in RLX is shown in **(Figure 3)**.

2.6 Bio-analytical Method

Bio-analytical method is one of the most useful method for the estimation of RLX in biological sample like tissue, Human plasma etc.A most advanced high throughput assay protocal was studied for quantification of tamoxifene, iodoxifene and raloxidene in human plasma with LC-MS/MS. Raloxifene was reported to impact on estrogen receptor activity in human skeletal muscle cells and may add benefits to muscle function. But the

It was also recommended that raloxifene might increase performance in athletes. But, raloxifene in urine sample of athletes has never been detected in annual overview of analytical results published by anti-doping laboratories published by WADA during 2003 to 2010.

Reports suggested few analytical methods such as LC-MS-MS for detection of raloxifene.

Most of these analytical methods are cumbersome and time consuming. The present review revealed the LC- MS method used for the determination of RLX. This is summarized in **Table 4.**

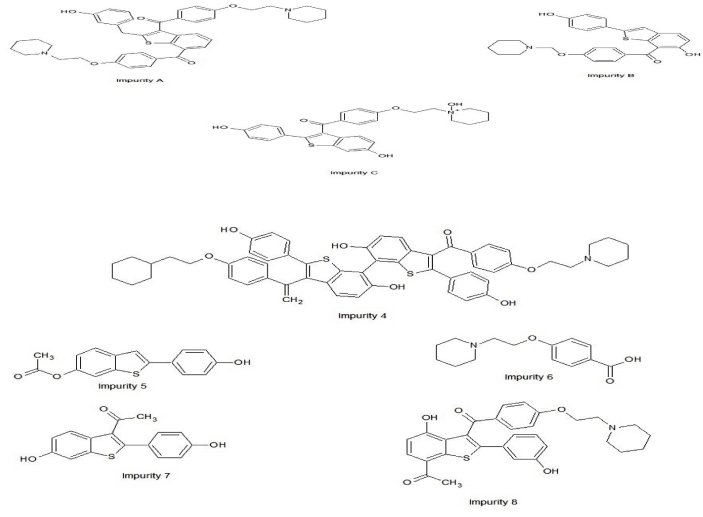

Table 3: RP-HPLC Methods for Analysis of RLX in Pharmaceutical dosage form.

Table 4: Bioanalytical LC-MS/MS Method.

Sr. No	Mobile Phase	Column	Partical Size Internal diameter	Mode	References
1.	A mixture of acetonitrile and 2 mM ammonium acetate containing 0.1% formic acid (70:30, v/v)	Cosmosil 5C_{18}-MS reversed-phase	50 × 4.6 mm, 5 μm,	Isocratic	42
2.	Fisher Chemical Optima formic acid, 90%, LC-MS grade	Thermo Scientific™ Hypersil GOLD™ PFP	100 × 3 mm, 3 µm	Gradient	43
3.	Mobile phase A 5mM Ammonium formate containing 0.1% formic acid and Mobile Phase B Acetonitrile	A Thermo hyper Gold C_{18} reversed-phase column	50X 2.1 mm, 3µm	–	44
4.	Mobile Phase A: 0.1% Formic acid in water and 100% Mobile Phase B: Acetonitrile	Kinetex column coupled with an In-Line filter KrudKatcher (Phenomenex, Torrance, USA)	50 ×2.1 mm 3µm 4 ×2 mm	Gradient	45
5.	Mobile phase A: 0.1% formic acid in acetonitrile and Mobile phase B : 0.1% formic acid in water	ProStar 210 liquid chromatography (Varian) using a Luna C_{18}(2),	50 × 2.0 mm 3µm	–	46

LC-MS method for estimation of RLX and its metabolite in human plasma has been studied. Two main glucuronide metabolite, raloxifene -6-glucuronide (M1) and raloxifene-4- glucuronide (M2) in human plasma has been studied.

Table 5: Stability-indicating HPLC and HPTLC methods

Sr. No	Name of Drug	Sample	Mobile Phase	Detection Wavelength (nm)	Linearity	Rt/Rf	r^2 value	Ref.
1	RLX	Bulk	Sodium acetate: Methanol (40:60 v/v)	287	1.0-250 μg/ml	5.1 ± 0.05	0.999	48
2	RLX	Bulk	Methanol: Water: Ammonia (9.4:0.5:0.1 v/v)	254	100-500 ng/band	0.60 ± 0.02	0.999	49
3	RLX	Bulk	Methanol: Water: Ammonia (95:05:0.1 v/v)	311	100-600ng/ band	0.55± 0.02	0.996	50
4	RLX	Bulk	Ethyl acetate: Methanol: Ammonia (7:3:0.1 v/v/v)	254	3-11 μg/ml	0.83	0.996	51

2.7. Capillary Electrophoresis

The migration nature of raloxifene was explored by capillary electrophoresis (CE). The influence of various parameters such as nature and concentration of the running buffer, pH and applied voltage on migration time, peak symmetry and efficiency was thoroughly investigated. 20mM acetate buffer of pH 4.5 was found to give a very stable electrophoretic system for the analysis of raloxifene. The plasma sample were also investigated by applying HPLC Beckman Coulter(Fullerton CA, USA) instrument. The chromatography was studied on a 5 um utrasphere C18 column using acetonitrile/10mM SDS (11.9, pH 7) and detection was done at 286 nm. [47]

2.8. Stability–Indicating Method (SIM) used for determination of RLX

Stability- indicating methods are quantitative test methods that can identify change in API and drug products throughout the time and under certain conditions. Food and Drug Administration (FDA), European Medicine Agency, other regulatory agencies, ICH and good manufacturing practices need establishment and validation of stability indicating method.

Five stability indicating methods have studied so far for determination of RLX in pharmaceutical formulation. The details about the stability indicating methods are summarized in **Table 5.**

CONCLUSION

The present review gives detailed account on analytical methods explored for the analysis of RLX. Various analytical techniques have been studied includes HPLC, HPTLC, Capillary electrophoresis, LC-MS for estimation of RLX in pharmaceutical formulation and biological fluids. Liquid chromatography with UV detection has been the most explored technique for quantification of RLX and RLX impurities in bulk as well as in pharmaceutical dosage form. Hyphenated technique such as LC-MS, LC-MS/MS are studied for estimation of RLX and its metabolite in human plasma. Few other methods such as HPLC, HPTLC and capillary electrophoresis have also been studied.

ACKNOWLEDGEMENT

Authors are thankful to a Principal of R.C. Patel Institute of pharmaceutical Education and Research, Shirpur Dist: Dhule (MS) 425 405 India for providing necessary library facilities.

REFERENCES

[1] Muchmore, D.B., (2000). Raloxifene: a selective estrogen receptor modulator (SERM) with multiple target system effects. The Oncologist, **5(5),** 388–392. https://doi.org/10.1634/theoncologist.5-5-388

[2] Tripathi, K.D., (2013). Essentials of medical pharmacology. JP Medical Ltd. https://doi.org/10.5005/jp/books/12256

[3] Pathuri, R., Muthukumaran, M., Krishnamoorthy, B. and Nishat, A., et al. (2013). A Review on Analytical Method Development and Validation of Pharmaceutical Technology. Current Pharma Research, **3(2),** 855.

[4] Connors, K. A., (1999). Liquid Chromatography- A Textbook of Pharmaceutical Analysis. New York: Willey Inter science.

[5] Backett, A. H., Stenlake, J. B. (1997) Practical Pharmaceutical Chemistry. 4thedn., Part II, CBS Publications and Distributors.

[6] Pharmacopoeia, (2014). Government of India Ministry of Health & Family Welfare The Controller & Publication. Delhi, India.

[7] Kalyanaramu, B., Raghubabu, K., Vamsi Kumar, Y. and JagannadhaRao, V., 2011. A novel method for estimation of raloxifene hydrochloride in bulk and pharmaceutical preparations by visible spectrophotometry. Der PharmaChemica, **3(2),** 250–256.

[8] Kalyanaramu, B. and Raghubabu, K., 2011. Development of new analytical method for determination of raloxifene hydrochloride in formulations based on charge-transfer complex formation. Int J Anal Bioanal Chem, **1(2),** 29–33.

[9] Dhumal Dinesh, PayalHasmukhlalPatil, Sanjaykumar Bari 2012 Quantitative determination of raloxifene hydrochloride in bulk and tablet formulation by UV-spectrophotometry and first order derivative using area under cuvrve method Ind Drugs 49(09)

[10] Nagaraju PT, K VenuGopal, Murali Krishna NV*, Bhargavi V, and Srinivasulu N 2014 UV - Spectrophotometric Method Development and Validation for Determination of Raloxifene in Pharmaceutical Dosage Form rrjpa

[11] Basavaiah, K., Anil Kumar, U.R., Tharpa, K. and Vinay, K.B., 2008. Validated spectrophotometric methods for the determination of raloxifene hydrochloride in pharmaceuticals. Journal of the Chilean Chemical Society, **53(3),** 1635–1639. https://doi.org/10.4067/S0717-97072008000300020

[12] Kalyanaramu, B. and Raghubabu, K., 2011. Determination of raloxifene hydrochloride by oxidative coupling reaction in pharmaceutical formulations. Int. J. Appl. Pharm, **3,** 6–9.

[13] Basavaiah, K. and Kumar, U.R., 2006. New sensitive spectrophotometric methods for the determination of raloxifene hydrochloride in pharmaceuticals using bromate-bromide, methyl orange and indigo carmine. Journal of Chemistry, **3(4),** 242–249. https://doi.org/10.1155/2006/472186

[14] Kalyanaramu, B. and Raghubabu, K., 2011. A quantitative assay for raloxifene hydrochloride in bulk and pharmaceutical preparations by visible spectrophotometry. J. Chem. Pharm. Res, **3(1),** 122–127.

[15] Salazar, F.R., Codevilla, C.F., Meneghini, L. and Bergold, A.M., 2015. Development of alternative methods for the determination of raloxifene hydrochloride in tablet dosage form. Brazilian Journal of Pharmaceutical Sciences, **51(2),** 349–360. https://doi.org/10.1590/S1984-82502015000200012

[16] Kalyanaramu, B., Raghubabu, K., Vamsi Kumar, Y. and JagannadhaRao, V., 2011. A novel method for estimation of raloxifene hydrochloride in bulk and pharmaceutical preparations by visible spectrophotometry. Der PharmaChemica, **3(2),** 250–256.

[17] Annapurna, M., Rao, E and Kumar, B.V.V., (2007). Spectrophotometric determination of raloxifene hydrochloride in pharmaceutical formulations.Journal of Chemistry, **4(1),** 79–82. https://doi.org/10.1155/2007/480625

[18] Moussa, A., El-Bagary, I and Osman, E.E.A., (2013). Optimized Spectrophotometric Methods for the Determination of Raloxifene Hydrochloride. International Research Journal of Pure and Applied Chemistry, **3(3),** 220. https://doi.org/10.9734/IRJPAC/2014/4588

[19] Pavithra, C and Lakshmi S., (2006). New Spectrophotometric Determination of Raloxifene Hydrochloride in Tablets. Indian Journal of Pharmaceutical Sciences, **68,** 3.

[20] Basavaiah K., Tharpa K., Anil Kumar R., Rajedraprasad N., HiriyannaSg., Vinay, B., (2009). Optimized and validated spectrophotometric methods for the determination of raloxifene in pharmaceuticals using permanganate. Arch Pharm Res., **32(9),** 1271–1279. https://doi.org/10.1007/s12272-009-1913-7

[21] Moussa, A., El-Bagary, I and Osman, E.E.A., (2013). Spectrophotometric and Chromatographic Methods for the Estimation of Raloxifene Hydrochloride in Bulk and Pharmaceutical Formulations. American Chemical Science Journal, **3(4),** 378–386. https://doi.org/10.9734/ACSJ/2013/4598

[22] Sathyaraj, A., Satyanarayana, V. and Basaveswara, R., 2011. Gradient RP-HPLC method for the determination of Purity and Assay of Raloxifenehydrochloride in Bulk Drug. Research Journal of Chemical Sciences, **1(2),** 9–16.

[23] Reddy, P.V., Rani, B.S., Babu, G.S. and Rao, J.V.L.N., 2006. RP-HPLC determination of Raloxifene in pharmaceuticl tablets. Journal of Chemistry, **3(1),** 60–64. https://doi.org/10.1155/2006/713569

[24] Pulla Reddy, S., Vardhan, S.V.M., Ravi Kumar, D., Shanmukha Kumar, J.V. and Rambabu, C., 2012. Development and Validation of RP-HPLC Method for the Estimation of Raloxifene in Marketed Formulations. Analytical Chemistry Letters, **2(6),** 358–362. https://doi.org/10.1080/22297928.2012.10662620

[25] Kumar, B.V., Kumar, K.P., Suresh, K., Aspar, S., Srikanth, P. and Suneetha, Y., 2011. Development and validation of RP-HPLC method for determination of raloxifene hydrochloride from pharmaceutical preparation. J Chem Pharm Res, **3(3),** 784–91.

[26] Sandeep K*, Vijaykumar N, Mohanvarma M and Veerabhadraswamy P 2013 Development and validation of a dissolution method for Raloxifene hydrochloride in pharmaceutical dosage forms using RP-HPLC J Chem Pharm Res, **5(12),** 981–987

[27] Madhu, B., Kumara, A.A., Prashanth, S., Kumar, Y., Pradeep, R.J. and Ramesh, G., 2011. Sensitive and rapid HPLC method for the determination of raloxifene hydrochloride. Journal of Pharmacy Research, **4(3),** 582–584.

[28] Trontelj, J., Vovk, T., Bogataj, M. and Mrhar, A., 2005. HPLC analysis of raloxifene hydrochloride and its application to drug quality control studies. Pharmacological research, **52(4),** 334–339. https://doi.org/10.1016/j.phrs.2005.05.007

[29] Mishra, R., Matkar, S., Malvia, H. and Khan, M.A., 2014. Development and Validation of a High Performance Liquid Chromatographic Method for Related Substances of Raloxifene Hydrochloride in Bulk and Pharmaceutical Formulations. Trends in Drug Delivery, **1(1),** 16–23.

[30] Basavaiah, K., Anil Kumar, U. and Tharpa, K., 2008. Gradient HPLC analysis of raloxifene hydrochloride and its application to drug quality control. Actapharmaceutica, **58(3),** 347–356. https://doi.org/10.2478/v10007-008-0018-z

[31] Suneetha, D. and Rao, A.L., 2010. A new validated RP-HPLC method for the estimation of raloxifene in pure and tablet dosage form. Rasayan Journal of Chemistry, **3 (1),** 117–121.

[32] Salazar, F.R., Codevilla, C.F., Meneghini, L. and Bergold, A.M., 2015. Development of alternative methods for the determination of raloxifene hydrochloride in tablet dosage form. Brazilian Journal of Pharmaceutical Sciences, **51(2),** 349–360. https://doi.org/10.1590/S1984-82502015000200012

[33] Pavithra, D.C. and Sivasubramanian, L., 2006. RP-HPLC estimation of raloxifene hydrochloride in tablets. Indian journal of pharmaceutical sciences, **68(3),** 401–402 https://doi.org/10.4103/0250-474X.26660

[34] Farmer, S., Anderson, P., Burns, P., and Velagaleti, R., (2002). Forced Degradation of Ibuprofen in Bulk Drugs and Tablets. Pharmaceutical Technology **28(42),** 13.

[35] Bhat, P. and Velingkar, VS., (2004). Synthesis and Characterization of Degradation Products in Diclofenac-Na and Clotrimazole. Indian Drugs, 40 (7): 396.

[36] Volk, KJ., Hill, SE., Kerns, EH., Lee MS., (1997). Profiling degradants of paclitaxel using liquid chromatography-mass spectrometry and liquid chromatography-tandem mass spectrometry substructural techniques. J Chromatogr B, **696(1),** 99. https://doi.org/10.1016/S0378-4347(97)00208-9

[37] European Pharmacopoeia Commission and European Directorate for the Quality of Medicines & Healthcare, 2010. European pharmacopoeia. Renouf Publishing Company Limited. 7.0, 284.2–2843.

[38] USP-NF27. Pharmacopeial Forum. **33(4),** 673.

[39] Jančić-Stojanović, B., Rakić, T., Kostić, N., Vemić, A., Malenović, A., Ivanović, D. and Medenica, M., (2011). Advancement in optimization tactic achieved by newly developed chromatographic response function: application to LC separation of raloxifene and its impurities. Talanta, **85(3),** 1453 1460. https://doi.org/10.1016/j.talanta.2011.06.029

[40] Jagadeesh, N., Kumara, Y.R., Jayashreeb, A. and Mohantya, S., (2014). Structural Elucidation of Potential Impurities of Raloxifene Hydrochloride by LC/ESI-MS and NMR. Journal of Pharmacy Research, **8(6),** 718–727.

[41] Reddy, BR., Goud, VT., Nagamani, n., Pavankumar, n., Alagudurai, A., Murugan, R., Parthasarathy, K., Karthikeyan, V. and Balaji, P., (2012). Identification and characterization of potential impurities in raloxifene hydrochloride. Scientiapharmaceutica, **80(3),** 605–618. https://doi.org/10.3797/scipharm.1204-13

[42] Chen, T.T., Huang, T.Y., Pan, R.N., Chang-Chien, G.P. and Hsu, M.C., (2013). Determination of Raloxifene in Urine by Liquid Chromatography–Tandem Mass Spectrometry for Doping. Journal of analytical toxicology, **37(6),** 345–350. https://doi.org/10.1093/jat/bkt041

[43] Krishna, D., Dr. Tushar, M.,LC-MS/MS Method for the Determination of Raloxifene and its Glucuronide Metabolites from Human Plasma Using SPE Micro Elution. Thermo scientific, 1–8.

[44] Kang, J., Hwang, H., Lee, W. and Kim, H., (2007). Validation and application of a screening method for β2−agonists, anti−estrogenic substances and mesocarb in human urine using liquid chromatography/tandem mass spectrometry. Rapid communications in mass spectrometry, **21(2),** 252–264. https://doi.org/10.1002/rcm.2834

[45] Trdan, T., Roškar, R., Trontelj, J., Ravnikar, M. and Mrhar, A., (2011). Determination of raloxifene and its glucuronides in human urine by liquid chromatography–tandem mass spectrometry assay. Journal of Chromatography B, **879(23),** 2323–2331. https://doi.org/10.1016/j.jchromb.2011.06.031

[46] Jurij, T., Marija, B., Janja, M., Aleˇs, M., (2007).Development and validation of a liquid chromatography–tandem mass spectrometry assay for determination of raloxifene and its metabolites in human plasma. Journal of Chromatography B, **855(2),** 220–227. https://doi.org/10.1016/j.jchromb.2007.05.004

[47] Perez-Ruiz, T., Martı´nez-Lozano, C., Sanz, A. and Bravo, E., (2004). Development and validation of a quantitative assay for raloxifene by capillary electrophoresis. Journal of pharmaceutical and biomedical analysis, 34(5), 891-897. https://doi.org/10.1016/j.jpba.2003.12.008

[48] Sowjanya, G., Annapurna, M. and RAO, S., (2012). Validated stability-indicating liquid chromatographic method for the determination of raloxifene (anti-osteoporotic agent) in tablets. Journal of Drug Delivery and Therapeutics, **2(4),** 4. https://doi.org/10.22270/jddt.v2i4.257

[49] Rajasekaran, A., Preethi, N., Arivukkarasu, R.,(2014). Stability indicating High Performance Thin Layer Chromatographic determination of Raloxifene Hydrochloride. Pharma Research Library, **2(2),** 348–355.

[50] Shirkhedkar, A., Rajput, K., Rajput, K. and Surana, S.J., (2012). Stability-Indicating RP-TLC/Densitometry Determination of Raloxifene Hydrochloride in Bulk Material and in Tablets. Chromatography Research International, 2012, 1–7. https://doi.org/10.1155/2012/356216

[51] Merey, A., Galal, M., Salem, Y. and Abdel-Moety, M., (2013). Novel stability indicating methods for the determination of certain synthetic estrogen level modifiers. Bulletin of Faculty of Pharmacy, Cairo University, **51(1),** 69–79. https://doi.org/10.1016/j.bfopcu.2012.11.001