Acta Pharm. *58* (2008) 347–356
10.2478/v10007-008-0018-z

Short communication

Gradient HPLC analysis of raloxifene hydrochloride and its application to drug quality control

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A rapid, sensitive and selective method for the determination of raloxifene hydrochloride (RLX) in pure drug and in tablets was developed using gradient high performance liquid chromatography (HPLC). The devised method involved separation of RLX on a reversed phase Hypersil ODS column and determination with UV detection at 284 nm. The standard curve was linear (R = 0.999) over the concentration range of 50–600 μg $mL^{-1}$with a detection limit of 0.04 μg mL^{-1} and a quantification limit of 0.16 μg mL^{-1}. Intra-day and inter-day precision and accuracy of the method were established according to the current ICH guidelines. Intra-day RSD values at three QC levels (250, 450 and 550 μg mL^{-1}) were 0.2–0.5%, based on the peak area. The intra-day relative error (e_r) was between 0.2 and 0.5%. The developed method was successfully applied to the determination of RLX in tablets and the results were statistically compared with those obtained by a literature method. Accuracy, evaluated by means of the spike recovery method, was the excellent with percent recovery in the range 97.7–103.2 with precision in the range 1.6–2.2%. No interference was observed from the coformulated substances. The method was economical in terms of the time taken and the amount of solvent used.

Keywords: raloxifene hydrochloride, gradient HPLC, pharmaceuticals

Accepted July 3, 2008

Raloxifene hydrochloride (RLX) is a selective estrogen receptor modulator that belongs to the benzothiophene class of compounds (Fig. 1) (1). Its chemical designation is methanone[6-hydroxy-2-(4-hydroxyphenyl)benzo[b]thien-3-yl]-[4-[2-(1-piperidinyl)ethoxy]phenyl]hydrochloride. RLX is used for prevention of osteoporosis in postmenopausal women.

A few HPLC methods with UV detection have been previously reported for the determination of RLX in pharmaceuticals (2–7). Other techniques reported for the assay of RLX in pharmaceuticals include UV and VIS spectrophotometry (8–10), capillary electro-

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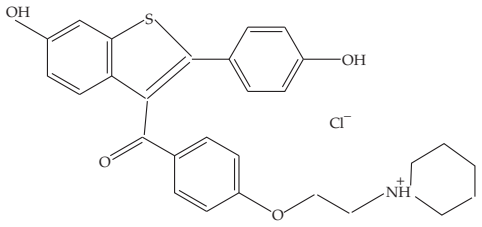

Fig. 1. Structure of raloxifene hydrochloride.

phoresis (11) and Rayleigh scattering (11). Some of the reported methods, however, suffer from such disadvantages as poor selectivity, sensitivity, accuracy and precision (Table I).

This paper deals with the development and validation of a sensitive gradient HPLC method for the assay of RLX in pharmaceuticals. Separation and determination were done on a reversed phase Hypersil C_{18} column and UV-detection at 284 nm.

EXPERIMENTAL

Apparatus

The chromatographic system consisted of an Agilent 1100 series chromatograph equipped with an in-built solvent degasser, quaternary pump, photo diode array detector with variable injector and auto sampler, and a reversed phase 5-μm Hypersil ODS column (250 × 4.6 mm i.d.).

Reagents and standards. – All chemicals used were of analytical reagent grade. Ammonium acetate and acetic acid (from s.d. Fine Chem. Ltd, India) and HPLC grade acetonitrile (from Merck. Ltd, India) were used. Distilled water filtered through a 0.45 μm filter (Millipore, India) was used to prepare solutions. A diluent consisting of 60% acetonitrile and 40% water (*V*/*V*) was used to prepare the sample preparations.

A 0.154% ammonium acetate (*m*/*V*) solution, pH adjusted to 4.0 with acetic acid, was the mobile phase A and acetonitrile was the mobile phase B.

Pharmaceutical grade RLX, certified to be 99.8% pure, was procured from Cipla India Ltd, India, and was used as received. For the study, an accurately weighed 50 mg of RLX was dissolved and diluted to the volume with the diluent solution in a 50 mL calibrated flask to obtain a concentration of 1000 μg mL^{-1} RLX.

Procedures

Chromatographic conditions. – Separation was achieved at ambient temperature on the column using the mobile phase at a flow rate of 1.2 mL min^{-1}. The detector wavelength was set at 284 nm with sensitivity of 0.2 a.u.f.s.

Gradient composition was as follows:

Time (min)	0	30	35	40	45
A (%)	50	10	10	50	50
B (%)	50	90	90	50	50

Table I. Comparison of the proposed HPLC method with the existing HPLC and other methods

Method	Experimental details	Detection	Linear range ($\mu g\ mL^{-1}$)	*QL*	Remarks	Ref.
HPLC	Octadecyl-bonded silica column; 3:7 acetonitrile/0.05 M ammonium acetate as mobile phase	UV at 286 nm	NA	NA	Application to related substances in RLX raw medicine.	2
HPLC	NA	UV	0.5–10	0.610 ($\mu g\ mL^{-1}$)	Less accurate and precise; –6.9–0.3; intra-day and inter-day RSD values were 2.38% and 7.93%, resp.	3
HPLC	Acetonitrile-0.01 M sodium dodecylsulphate (55:45, pH 4.0) was the mobile phase	UV at 286 nm	NA	NA	–	4
HPLC	Merck RP-18 cartridge octadecylsilane was the stationary phase; acetonitrile/water (40:60, pH 3.0) was the mobile phase (1 mL min^{-1})	UV at 225 nm	250–750	–	Less sensitive; narrow linear dynamic range; less precise (RSD > 0.8%).	5
HPLC	Intersil C_{18} column; acetonitrile-phosphate buffer (pH 2.0) was the mobile phase (1 mL min^{-1})	UV at 280 nm	0.5–200	NA	Less precise, intra-day and inter-day RSD >1%.	6
RP-HPLC	Waters symmetry C_{18}; Methanol-water (50:50 *V/V*) was the mobile phase (1 mL min^{-1})	UV-230 nm	10–60 mg mL^{-1}	4.04 mg mL^{-1}	Least sensitive	7
UV-spectropho--tometry	NA	NA	NA	NA	Less accurate and precise, recovery, 99.32% with RSD of 0.895.	8

Visible spectrophotometry	*(i)* $FeCl_3$-ferricyanide *(ii)* Fehling regent	735 nm	NA	NA	Prone to interference by reducing sunbstances.	9
Visible spectrophotometry	*(i)* 0.1 M-NaOH *(ii)* $FeCl_3$-O-phen. *(iii)* $FeCl_3$-bipyridyl	425 nm 510 nm 521 nm	5–150 1–10 2–25	– – –	Measurement at shorter wavelength where the interference from excipients is serious. Involves heating at 60 °C for 15 min.	10
Capillary electrophoresis	pH 4.5 buffer consisting of 20 mM sodium acetate was used	–	–	–	Migration behavior and hence results dependent on too many variables	11
Resonance Rayleigh scattering (RRS)	Intensity of RRS of the ion-association complex formed b/n RLX and evans blue in NaAc-HCl buffer for (pH 1.8) measured	–	0–8.3	18.9 ng mL^{-1} (*DL*)	Not very selective	12
HPLC (gradient)	Hypersil ODS column; ammonium UV-at acetate (pH 4.0) and acetonitrile were the two mobile phases used (1.2 mL min^{-1})	UV-at 284 nm	50–600	0.16 μg mL^{-1}	Wide linear dynamic range, highly precise (intra-day and inter-day RSD < 0.5%) and accurate (e_r < 0.5%)	This paper

NA – not available

Calibration. – Working standard solutions equivalent to 50 to 600 μg mL^{-1} RLX were prepared by appropriate dilution of the stock standard solution (1000 μg mL^{-1}) with the diluent solution. Twenty-μL aliquot of each solution was injected automatically onto the column in duplicate and the chromatograms were recorded. Calibration graph was prepared by plotting the mean peak area *vs.* RLX concentration.

The concentration of the unknown was read from the calibration graph or computed from the regression equation derived from the mean peak area-concentration data.

Assay in dosage forms. – The following formulations containing RLX were purchased from local commercial sources and used in the investigation: A, Fiona tablets (Dr. Reddys Ltd, India) containing 60 mg of RLX, B, Gynista tablets (Micro Nova Ltd, India) containing 60 mg of RLX per tablet and C. Ronal tablets (Blue Cross Ltd, India) containing 60 mg of RLX.

A quantity of tablet powder equivalent to 100 mg of RLX was accurately weighed into a 100 mL calibrated flask, 60 mL of diluent solution was added and the content was shaken for 20 min; the volume was then diluted to the mark and mixed well. A small portion of the extract (say, 10 mL) was withdrawn and filtered through a 0.2-μm filter to ensure the absence of particulate matter. The filtered solution was appropriately diluted with the diluent solution for analysis as already described.

Recovery experiment. – To a fixed and known amount of the drug in tablet powder (pre-analyzed), pure RLX was added at three different levels, and the total was found by the proposed methods from which the percent recovery of pure drug added was calculated.

Selectivity testing. – A separate selectivity test was performed by applying the proposed methods to the determination of RLX in a synthetic mixture consisting of RLX, talc, starch, lactose, calcium gluconate, calcium dihydrogenorthophosphate, sodium alginate and magnesium stearate, in the mass ratio of 1: 2.5: 3.0: 0.3: 0.5: 0.2: 0.7: 1 RLX was extracted with three 20-mL portions of diluent and filtered. The filter was washed with diluent; the filtrate and washings were collected in a 100-mL calibrated flask and diluted to the volume with diluent and mixed well. An appropriate aliquot of the extract was subjected to analysis as stated earlier.

RESULTS AND DISCUSSION

Method development

A solution of RLX was injected in duplicate onto the column and was monitored by UV-detection at 284 nm. A gradient method was selected rather than an isocratic one to get faster elution with less retention time. At a flow rate of 1.2 mL min^{-1}, the retention time was 6.65 min (Fig. 2a). Under the described experimental conditions, the peak was well defined and free from tailing. RLX was determined by measuring the peak area. Plot of the mean peak area against concentration gave the linear relationship ($R = 0.9991$, $N = 5$) over the concentration range 50–600 μg mL^{-1}. Using the regression analysis, the linear equation, $Y = -184.40 + 57.88\,\gamma$ was obtained, where Y is the mean peak area and γ is concentration in μg mL^{-1}. The limits of detection and quantification calculated according to ICH guidelines were 0.04 and 0.16 μg mL^{-1}, respectively (13).

Method validation

In order to determine the adequate resolution and reproducibility of the method, suitability parameters, including retention time, plate number and tailing factor, were investigated and were found to be 6.65 min, 4047 and 1.72, respectively, which amply demonstrates the method suitability. Retention time varied for 0.2%.

Specificity. – Specificity of an analytical method may be defined as the ability to unequivocally determine the analyte in the presence of additional components such as impurities, degradation products and matrix (13–15). Specificity was evaluated by preparing an analytical placebo and it was confirmed that the signal measured was caused only by the analyte. A solution of analytical placebo (containing all the tablet excipients except RLX) was prepared according to the sample preparation procedure and injected.

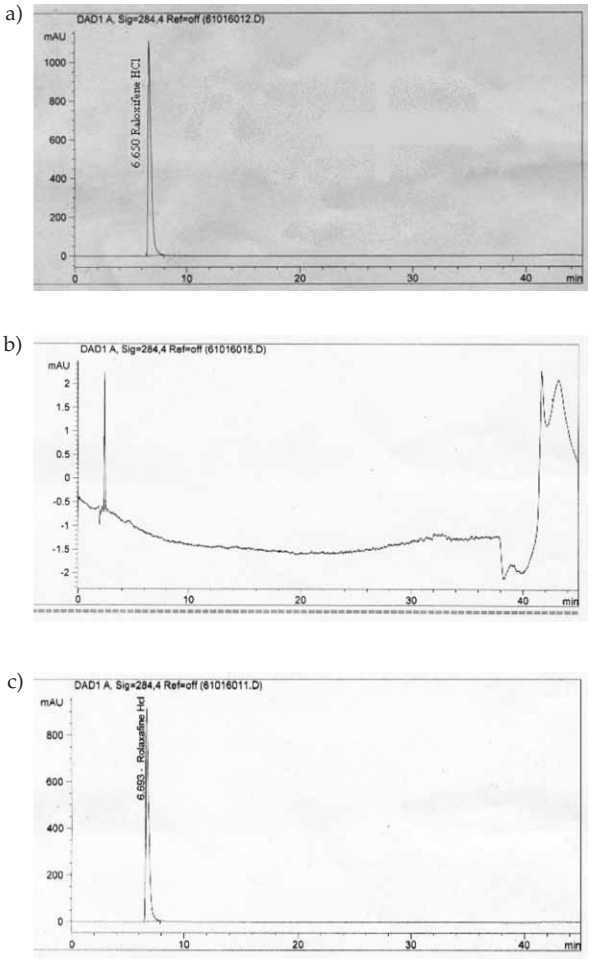

Fig. 2. Chromatograms: a) pure RLX, b) placebo and c) tablet extract (300 μg mL^{-1} RLX).

The resulting chromatogram is shown in Fig. 2b. To identify the interference by these excipients, the tablet extract after appropriate dilution was chromatographed. The resulting chromatogram did not show any peak other than that of RLX (Fig. 2c), which confirmed the specificity of the method. In addition, the slope of the calibration curve for standards was compared with that prepared from the tablet extract. It was found that there was no significant difference between the slopes, which indicated that excipients did not interfere with RLX.

Precision. – Precision of the method was evaluated in terms of intra-day and inter-day precision (12–14). Three different concentrations of RLX were analyzed in seven replicates on the same day (intra-day precision) and in five consecutive day (inter-day precision). Within each series, every solution was injected in triplicate. The peak-area based intra-day RSD values were 0.2–0.5%. The results of the study compiled in Table II are quite satisfactory. The inter-day precision showed somewhat higher RSD values of 0.6–0.9%. Intermediate precision was also evaluated by calculating the RSD values of six replicate determinations performed in standard RLX solutions by three different analysists with two different instruments. The inter-analyst RSD values were in the range 1.1–1.4% where the inter-instrument RSD values were 1.0 and 1.1%, respectively, for the two instruments used.

Accuracy. – Accuracy of an analytical method expresses the closeness between the reference value and the found value (12–14). The results obtained for e_r at three concentrations (within the linear range) are shown in Table II and were 0.2 to 0.5%. Accuracy was assessed by analyzing the synthetic mixture (prepared by adding RLX to the placebo) as described earlier. The calculated percent recovery of the active ingredient was found to be 99.6 ± 0.8 (n = 5) indicating that the co-formulated substances did not interfere with the assay.

Robustness. – Robustness of the method was checked by deliberately altering two critical parameters by minor variations: the flow rate was changed from 1.2 mL min^{-1} to 1.1 mL min^{-1} and the pH of the mobile phase A was changed from 4.0 to 3.9. The differences in the retention time and peak area (for a given RLX concentration) caused by the above minor alterations were insignificant.

Application

The developed and validated method was applied to the determination of RLX in three brands of tablets, each containing 60 mg per tablet. Evaluation was performed using the calibration curve method, since no significant difference between the slopes of the calibration curves for standards and tablet extracts was observed. The results obtained by the proposed method were statistically compared with those of the literature (UV-spectrophotometry) method (8) by applying Student's t-test for accuracy and F-test for precision. As shown by the results compiled in Table III, the calculated t- and F-values did not exceed the tabulated values at the 95% confidence level for four degrees of freedom, suggesting that the proposed method and the literature method did not differ significantly with respect to accuracy and precision.

The accuracy and validity of the proposed methods were further ascertained by performing recovery experiments. Pre-analyzed tablet powder was spiked with pure RLX

Table II. Relative error and intra-day precision

RLX taken (μg mL^{-1})	RLX found (μg mL^{-1})[a]	e_r (%)	RSD (%)[b]
250	249.5	0.2	0.4
400	398.2	0.5	0.5
550	548.6	0.3	0.2

[a] Mean value of seven determinations.
[b] Based on peak area.

Table III. Determination of raloxifene hydrochloride in tablets and comparison with the reference method

Formulation	Nominal amount (mg)	Found (%)[a]			
		Literature method (8)	Proposed method	*t*-value	*F*-value
A	60	101.3 ± 0.6	100.8 ± 1.3	0.83	4.33
B	60	98.2 ± 1.1	96.9 ± 1.5	1.62	1.95
C	60	101.5 ± 0.9	102.8 ± 1.6	1.67	3.59

[a] Mean ± SD, $n = 5$.
Tabulated *t*-value at 95% confidence level is 2.77.
Tabulated *F*-value at 95% confidence level is 6.39.

at three different levels and the total was found by the proposed methods. Each determination was repeated three times. Recovery of the pure drug added was in the range 97.7–103.2%, with the RSD values of 1.6–2.2%. The results of this study given in Table IV reveal that the common tablet excipients did not interfere with the determination.

The main features of the method are its wide linear dynamic range, high sensitivity, as shown by the *LOQ* value, and high accuracy and precision, as revealed by the recovery study and intra-day and inter-day precision studies.

Table IV. Recovery study

Formulation studied	RLX in formulation (μg mL^{-1})	Pure RLX added (μg mL^{-1})	Total found (μg mL^{-1})	Pure RLX recovered (%)[a]
	201.6	50	251.75	100.3 ± 1.7
A	201.6	200	408.00	103.2 ± 1.9
	201.6	350	550.90	99.80 ± 2.1
	205.6	50	255.45	99.7 ± 1.8
C	205.6	200	401.00	97.7 ± 1.6
	205.6	350	560.15	101.3 ± 2.2

[a] Mean ± SD, $n = 3$.

CONCLUSION

In conclusion, a gradient reversed-phase HPLC-UV assay was developed for the determination of raloxifene and validated as per the current ICH guidelines. The method is simple, precise and accurate, selective and sufficiently sensitive compared to many similar methods reported earlier (Table I). Hence, it seems suitable for the determination of the drug either in bulk or in tablets without interference from commonly used excipients and could be used in a quality control laboratory.

Acknowledgments. – Two of the authors (Urdigere Rangachar Anil Kumar and Kalsang Tharpa) thank the authorities of the university of Mysore, Mysore, for research facilities. One of the author (Kalsang Tharpa) also thanks the Department of Education, Central Tibetan Administration of His Holiness the Dalai Lama, for providing a research scholarship.

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S A Ž E T A K

Gradijentna HPLC analiza raloksifen hidroklorida i primjena u kontroli kvalitete

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Koristeći gradijentnu tekućinsku kromatografiju visoke učinkovitosti razvijena je brza, osjetljiva i selektivna metoda za određivanje raloksifen hidroklorida (RLX), čiste supstancije i u tabletama. U radu je primijenjena reverzno-fazna kolona Hypersil ODS te UV detekcija pri 284 nm. Standardna krivulja bila je linearna (R = 0,999) u koncentracijskom području 50–600 μg mL^{-1}. Granica detekcije bila je 0,04 μg mL^{-1} a granica određivanja 0,16 μg mL^{-1}. Repetabilnost, intermedijalna preciznost i ispravnost ispitivane su prema važećim ICH uputama. Mjerenjem površine ispod pika na tri koncentracijske razine (250, 450 i 550 μg mL^{-1}) procijenjena je repetabilnost na 0,2–0,5%. Relativna pogreška procijenjena unutar jednog dana (e_r) bila je između 0,2 i 0,5%. Razvijena metoda uspješno je primijenjena za određivanje RLX u tabletama. Rezultati su statistički uspoređeni s rezultatima dobivenim prema ranije objavljenoj metodi. Analitički povrat bio je u rasponu 97,7–103,2 uz preciznost od 1,6 do 2,2%. Nije primijećena interferencija pomoćnih tvari. Metoda je ekonomična s obzirom na utrošeno vrijeme i količine upotrebljenog otapala.

Ključne riječi: raloksifen hidroklorid, gradijentna HPLC, ljekoviti pripravci

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