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Method development and validation for the HPLC potency assay of troglitazone tablets

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Abstract

This paper describes the development and validation of an isocratic, reversed-phase, high performance liquid chromatographic (HPLC) method for the assay of 200-mg troglitazone tablets. The chromatographic conditions of the method employ a YMC ODS-A, 120 Å (4.6 × 150 mm, 5 μm) column, isocratic elution with (50 mM aqueous NaH_2PO_4, pH 4.0):acetonitrile:methanol, (35:50:15, v/v/v) as the mobile phase at a flow rate of 1.0 ml/min, a 10 μl injection volume, and ultraviolet (UV) detection at 225 nm. The active was analyzed at ambient column temperature, using peak area responses. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Troglitazone [1] is an oral antihyperglycemic drug, whose action has been attributed to the decrease of insulin resistance. It is an agent that lowers blood glucose by improving target cell response to insulin in muscle and adipose tissue and inhibits hepatic gluconeogenesis. Troglitazone (±-5-[[4-[(3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-2H-1-benzopyran-2-yl)methoxy]phenyl]methyl]2,4-thiazolidinedione) belongs to a new class of compounds called thiazolidinediones that are chemically or functionally unrelated to any of the sulfonylureas, biguanides, or α-glucosidase inhibitors used for the treatment of diabetes. Only a few HPLC methods have been reported in the past, primarily for the analysis of troglitazone in human serum and plasma [2–4]. A new method was developed and validated for the assay of the drug in tablets. The structure of troglitazone is shown in Fig. 1.

2. Experimental

2.1. Reagents

HPLC grade acetonitrile (ACN), methanol (MeOH), and sodium phosphate, monobasic (monohydrate), AR grade, from Mallinckrodt

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(Phillipsburg, NJ); phosphoric acid (85%), AR grade; sodium hydroxide, AR grade; concentrated hydrochloric acid (HCl) 37%; hydrogen peroxide (H_2O_2), 30 and appropriate pH buffers from Fisher Scientific (Suwanee, GA); in-house picopure (HPLC) water. Troglitazone reference standard was synthesized and qualified in-house by the following methods: FT-IR, melting point (by DSC and capillary method), weight loss (by TGA), water content (by Karl Fisher), sulphated ash, heavy metals as Pb (USP method), and HPLC purity (by % Area). The analytical sample (Rezulin®, 200-mg tablets) was obtained from Parke Davis (New York, NY).

2.2. Equipment

The HPLC was a Hewlett Packard system (Palo Alto, CA), 1050 series, with HP CHEMSTATION DATA ACQUISITION software, versions A.02.05 and A.03.02, and a Hewlett Packard 1050 photodiode array detector. A Mettler AG245 analytical balance (Columbus, OH), a Branson 8210 sonicator (Danbury, CT) and a Beckman CS6K centrifuge (Fullerton, CA) were used. Gelman Acrodisc 0.45 μm CR PTFE (25 mm) membrane syringe filters (Ann Arbor, MI) and an Orion model 5208 pH meter (Beverly, MA) were also utilized.

2.3. Method development

Reverse phase chromatography was primarily evaluated using a variety of analytical HPLC columns, such as Hypersil ODS, 5 μm (15 cm × 4.6 mm) from Alltech (Deerfield, IL) and Novapak C18, 4 μm (15 cm × 3.9 mm) from Waters (Milford, MA) and a phosphate buffer in combination with acetonitrile or methanol as the mobile phase. The pH of the buffer solution in the mobile phase was 6–7 and 100% ACN or 100% MeOH was used each time as the organic component of the eluent. However, the chromatography, and especially the troglitazone peak shape, obtained under these conditions was unacceptable. The column was then changed to a YMC ODS-A (Wilmington, NC), 120 Å, 5 μm (15 cm × 4.6 mm) using a phosphate buffer at a lower pH of 4.0 as the aqueous component of the mobile phase with different ratios of acetonitrile–methanol mixtures. A composition of (35:50:15, v/v/v) of phosphate buffer–acetonitrile–methanol, produced no adverse chromatographic effects with an active retention time of approximately 10 min.

The selected extraction solvent was a mixture of water–acetonitrile (10:90, v/v), since the reference standard is very soluble in organic solvents and insoluble in water, whereas Rezulin® tablets disintegrate rapidly in water, and mobile phase was selected as the diluent for the final sample and standard dilutions.

An ultraviolet scan of a troglitazone reference standard solution in acetonitrile showed a stable absorbance maximum at 225 nm, which resulted in a robust detection and was chosen as the detection wavelength.

2.4. Preparation of mobile phase

The mobile phase was composed of a buffer solution (50 mM sodium phosphate monobasic in water (pH 4.0)–acetonitrile–methanol (35:50:15,

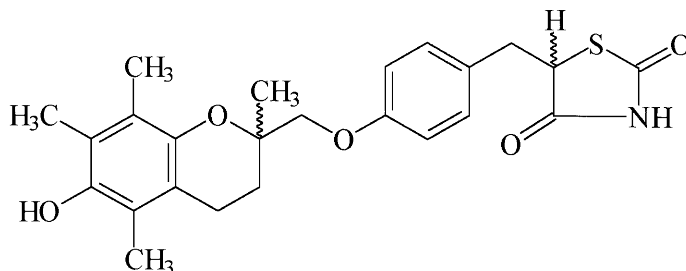

Fig. 1. Structure of troglitazone.

v/v/v)). The buffer solution was prepared by dissolving 6.9 g of sodium phosphate, monobasic (monohydrate) in 1 l of water and the pH was adjusted to 4.00 ± 0.05 with 85% phosphoric acid. For the preparation of the mobile phase, 350 ml of the buffer solution, 500 ml acetonitrile, and 150 ml methanol were combined, mixed well, allowed to equilibrate to room temperature, and degassed by helium sparge prior to use.

2.5. Preparation of standard extraction solvent

A mixture of water–acetonitrile (10:90, v/v) was prepared by combining 100 ml of water and 900 ml of acetonitrile.

2.6. Preparation of standard solution

A working standard solution at a concentration of approximately 0.20 mg/ml of troglitazone in mobile phase was prepared in the following manner:

Approximately 25 mg of troglitazone reference standard was accurately weighed and transferred into a 25-ml volumetric flask. Approximately 20 ml of standard extraction solvent was added and the solution was sonicated for 5 min or until the standard dissolved completely. After filling the flask to volume with standard extraction solvent and mixing well, a 5.0-ml portion of the resulting stock standard solution was transferred to a 25-ml volumetric flask, filled to volume with mobile phase, and mixed well.

2.7. Assay sample preparation

A composite of not less than ten tablets was prepared by grinding them to a fine, uniform particle size powder using a mortar and pestle. After calculating the average tablet weight, a composite equivalent to the average tablet weight (approximately 513 mg) was accurately weighed and quantitatively transferred into a 200-ml volumetric flask. Approximately 20-ml picopure water was added, the solution was sonicated for 10 min, 140 ml acetonitrile was added to it, and mechanically shaken for 10 more min. The flask was equilibrated to room temperature, carefully filled to volume with acetonitrile, and mixed well. A portion of the solution was filtered through a Gelman Acrodisc CR PTFE 0.45-μm filter, discarding the first 2–3 ml of the filtrate. A portion of the filtered sample (5.0 ml) was diluted into a 25 ml volumetric flask with mobile phase and mixed well.

2.8. Chromatographic conditions

A YMC ODS-A (15.0 cm × 4.6 mm, 5 μm) column was used at ambient temperature, with UV detection at 225 nm, injection volume of 10 μl and a flow rate of 1.0 ml/min. As mentioned in a previous section, the mobile phase consisted of a buffer solution (50 mM sodium phosphate monobasic in water (pH 4.0)–acetonitrile–methanol (35:50:15, v/v/v)). The peak area responses were used for quantitation, and the approximate retention time of troglitazone was 10 min (Fig. 2).

3. Results

3.1. Detectability (LOQ/LOD)

Troglitazone placebo samples, spiked with diluted reference standard were prepared. The dilutions were targeting active concentrations that would result in signal to noise ratios in the range of 8–15:1 for the limit of quantitation (LOQ) and 2–5:1 for the limit of detection (LOD). A troglitazone concentration of 0.20 μg/ml (0.1% of the label claim) resulted in an approximate signal-to-noise ratio of 10.6:1 (LOQ). The accuracy for the LOQ solution was 113.5% with a reproducibility of 9.7% (R.S.D.) for triplicate injections at this level.

The limit of detection (LOD) was determined to be 0.050 μg/ml with a signal-to-noise ratio of 3.0:1.

3.2. Range of linearity

The linearity of peak area responses versus concentrations was studied from approximately 0.10 to 0.30 mg/ml for troglitazone. This concen-

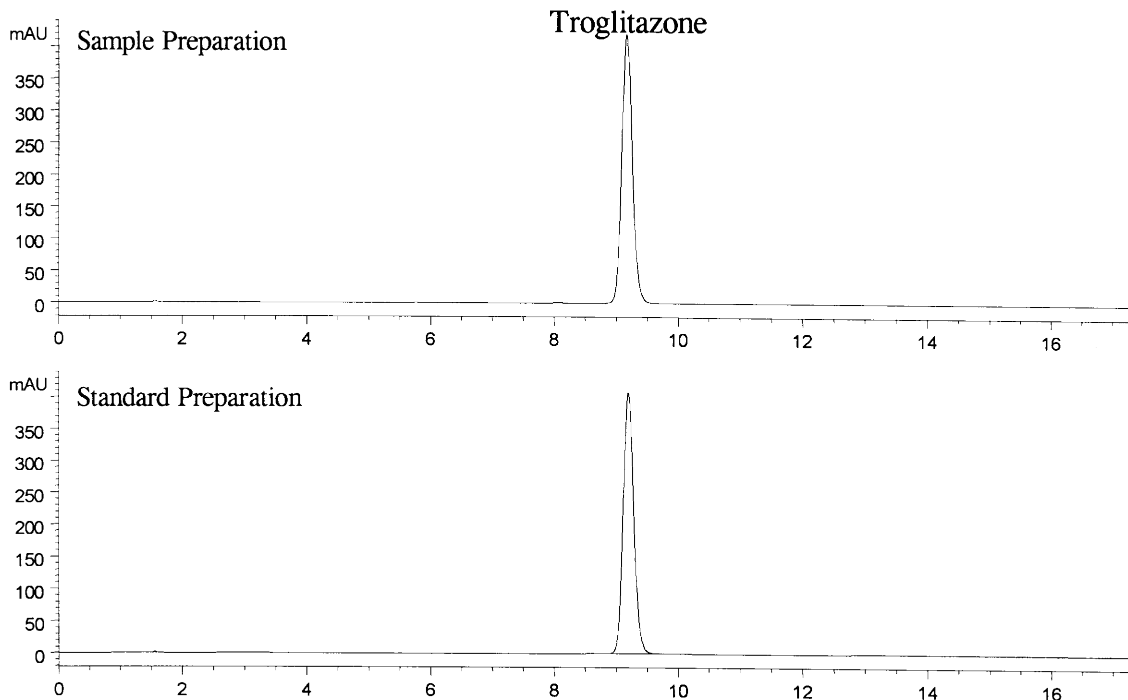

Fig. 2. Example chromatograms of standard and sample preparations.

Table 1
Range of linearity of troglitazone[a]

% w/w	Concentration (mg/ml)	Measured response	Calculated response	Residual	Response factor
50	0.0977	2436	2441	−4.9	24 920
		2432	2441	−9.0	24 880
80	0.156	3923	3913	10.2	25 090
		3920	3913	7.4	25 070
100	0.195	4904	4894	9.6	25 090
		4899	4894	5.2	25 070
120	0.235	5867	5876	−8.5	25 010
		5865	5876	−1.1	25 000
150	0.293	7359	7348	1.1	25 100
		7338	7348	−9.9	25 030

[a] y intercept, −13.2; slope, 25 106.6; correlation coefficient, 0.99999; % y-intercept, −0.3.

tration range corresponds to the approximate levels of 50–150% w/w of the nominal analytical concentration. The data (Table 1) meet the acceptance criteria for a correlation coefficient ≥ 0.999 and a y-intercept less than $\pm 2.0\%$.

3.3. System repeatability

The repeatability of the troglitazone peak area response was assessed from six replicate injections of a working standard and a sample solution at

the analytical concentration of about 0.2 mg/ml. The R.S.D. for the troglitazone response was found to be 0.1% for the standard solution and 0.2% for the sample solution.

3.4. Method repeatability/intermediate precision

Method repeatability/intermediate precision was assessed by the assay of three six-sample sets by two different analysts using different chromatographic systems on different days. The results are summarized in Table 2. The assay method repeatability/intermediate precision acceptance criteria set in the validation were that for each data set and for all the data combined the potency is within $100 \pm 2.0\%$ of the label claim (200 mg/tablet) with an R.S.D. $\leq 2.0\%$. The data of Table 2 met these acceptance criteria.

Table 2
Method repeatability/intermediate precision for troglitazone

Sample	% Label claim		
	Analyst #1	Analyst #2	
	Set #1	Set #2	Set #3
1	100.2	97.2	99.2
2	100.3	99.4	98.0
3	100.7	97.0	98.8
4	100.2	98.6	98.5
5	99.9	98.1	98.7
6	99.8	99.0	99.2
Mean (6)	100.2	98.2	98.7
% R.S.D.	0.3	1.0	0.5
Mean (18)	99.0		
% R.S.D.	1.1		

Table 3
Accuracy/recovery for troglitazone

Sample	% Recovery	Mean (3)	%R.S.D.	Level (%)
1	100.3	100.3	0.0	50
2	100.3			
3	100.3			
4	100.0	100.0	0.1	100
5	100.1			
6	99.9			
7	100.0	100.0	0.1	150
8	99.9			
9	100.0			
Mean (9)	100.1			
% R.S.D.	0.2			

3.5. Accuracy/recovery

The excipients in the Rezulin® tablets used in this validation study contained the following inactive ingredients: croscarmellose sodium, hydroxypropyl methylcellulose, magnesium stearate, microcrystalline cellulose, polyethylene glycol 400, polysorbate 80, povidone, purified water, silicon dioxide, titanium dioxide, and synthetic iron oxides. Placebo sample preparations were spiked with troglitazone reference standard (dry addition) at three different levels, corresponding to 50, 100, and 150% of the nominal analytical concentration of 0.20 mg/ml, with triplicate preparations at each level. The mean recovery data obtained for each level as well as for all levels combined (Table 3) were within 2.0% of the label claim for the active with an R.S.D. $\leq 2.0\%$, which satisfied the acceptance criteria set for the study.

3.6. Specificity

Injections of diluent (mobile phase) and placebo tablet solutions showed no interference with the elution of troglitazone (Fig. 3).

3.7. Filter study

The filtration process of the method was qualified by comparing six separately filtered portions of an assay sample preparation against six portions of the same solution that were clarified by centrifugation for 10 min at 3000 rotations per min. The acceptance criteria for the filtration study were that the mean recovery of the filtered relative to the centrifuged aliquots should be $100.0 \pm 1.5\%$ with an R.S.D. ≤ 2.0. The data of Table 4 met the acceptance criteria for the filtration study.

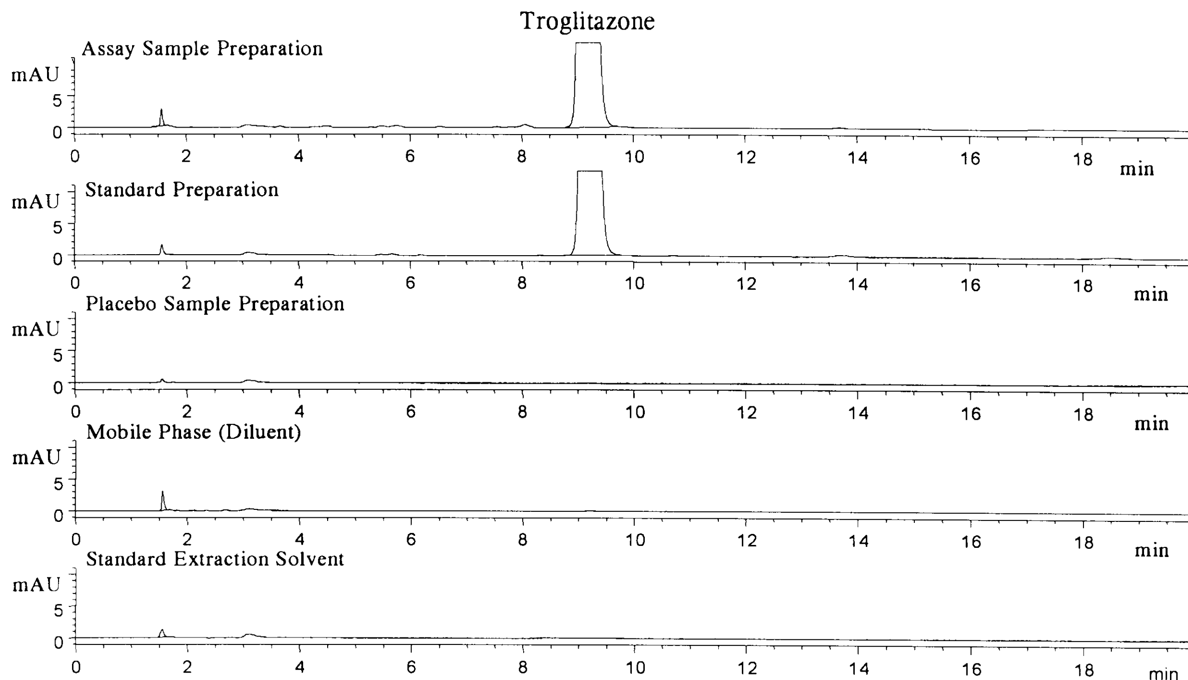

Fig. 3. Chromatographic overlay of troglitazone sample, standard, extraction solvent, mobile phase, and a placebo solution.

3.8. Stability of analytical solutions

The stability of stock and working standard and sample solutions of troglitazone was assessed by the assay of the corresponding sample solutions immediately after their preparation and then, against freshly prepared standards, as they aged for 8 days at ambient laboratory conditions. The data of Table 5 met the acceptance criteria for an 8-day period.

3.9. Degradation studies

Forced degradation studies were performed to provide an indication of the stability-indicating properties and specificity of the method. The degradation samples were prepared by transferring sample composite, equivalent to the average tablet weight (approximately 513 mg), into 200 ml volumetric flasks. Intentional degradation was attempted using acid, base, hydrogen peroxide, heat, and light. After the degradation treatments were completed, the samples were allowed to equilibrate to room temperature and prepared according to *assay sample preparation*, after being neutralized with acid/base (when necessary). The

Table 4
Comparison of filtered and centrifuged samples

Preparation	Peak area		Filtered sample
	Centrifuged	Filtered	% Recovery[a]
1	5555.2	5576.1	100.1
2	5584.3	5574.7	100.1
3	5576.9	5591.5	100.4
4	5583.4	5588.2	100.3
5	5576.5	5584.0	100.2
6	5551.5	5591.2	100.4
Mean (6)	5571.3	5584.3	100.2
% R.S.D.	0.3	0.1	0.1

[a] % Recovery for the filtered samples was calculated based on the average troglitazone peak area response in the centrifuged samples.

Table 5
Stability of troglitazone in analytical solutions[a]

Day(s)	#1	#2	Mean (2)
Stability of sample solutions (% initial potency)			
Stock sample			
0	100.0	100.0	100.0
6	101.1	100.8	101.0
8	99.8	99.9	99.9
Working sample			
0	100.0	100.0	100.0
6	100.4	100.6	100.5
8	99.5	99.7	99.6
Stability of standard solutions (% standard check)			
Stock standard			
0	N/A	N/A	N/A
6	100.7	100.4	100.6
8	99.8	99.6	99.7
Working standard			
0	N/A	N/A	N/A
6	100.4	100.3	100.4
8	99.6	99.4	99.5

[a] Acceptance criteria: potency of aged preparation, fresh ± 2.0%.

samples were analyzed against a freshly prepared control sample (with no degradation treatment). The percent recovery of troglitazone is shown in Table 6. Degradation peaks, where observed, were resolved from the active peak. Spectra taken during the upslope, apex, and downslope did not reveal any degradation products or impurities coeluting with the active.

4. Conclusions

The linearity of the troglitazone peak area responses was demonstrated, from approximately 50 to 150% of the analytical concentration of 0.20 mg/ml, by a correlation coefficient of 0.999986 and a % y-intercept of -0.3%. The precision of troglitazone chromatographic peak area responses, calculated from five replicate injections of a tablet sample and a working standard solution showed R.S.D. of 0.2% (sample) and 0.1% (standard). Method precision was performed by assaying six product samples by two different analysts (a total of three different sets) on different days. The mean % label claim was 100.2% (R.S.D. = 0.3%) for the first set (analyst # 1), and 98.2% (R.S.D. = 1.0%) and 98.7% (R.S.D. = 0.5%) for two more sets (analyst # 2). The mean % label claim value for all eighteen sample preparations was 99.0 (R.S.D. = 1.1%). Working standard and sample solutions were both found stable for 8 days, under ambient laboratory conditions. Recoveries for triplicate preparations at levels corresponding to approximately 50, 100, and 150% of the nominal analytical concentration of 0.20 mg/ml troglitazone in tablet samples were 100.1% (R.S.D. = 0.2%) as a mean of all three levels. A filtration study demonstrated that a Gelman Acrodisc PTFE 0.45 μm filter is suitable for the filtration process of the method. Forced degradation studies on sample preparations did not exhibit any degradation peaks that would interfere with the elution of troglitazone.

Table 6
Degradation of troglitazone in 200 mg Rezulin® tablets

Conditions	Time (days)	% Recovered	Relative retention time of degradation products
Acid 1.0 N HCl, room temperature	3	100.5	0.62
Base 1.0 N NaOH, room temperature	40 min	88.2	0.37, 0.47, 0.49, 0.53, 0.55, 0.60, 0.63, 0.71, 0.74, 0.79
Hydrogen peroxide 5%, room temperature	2	96.3	0.47, 0.49, 0.60, 0.62, 0.73
Heat dry, 80°C	3	99.1	None detected
Heat wet, 80°C	3	99.3	0.59, 1.07
Light dry, 1000 foot candles, room temperature	5	102.3	None detected
Light wet, 1000 foot candles, room temperature	5	99.2	0.59

References

[1] The Merck Index, twelfth ed., Merck, 1996, pp. 1663–1664.

[2] H.K. Jajoo, N.V. Rao Mamidi, K. Kasiram, A.S. Prakash, V.V. Swaroop Kumar, P. Bheema Rao, V. Bhushan, S. Subramaniam, J. Chromatogr. B Biomed. Sci. Appl. 707 (1–2) (1998) 241–246.

[3] C.M. Loi, E.J. Randinitis, A.B. Vassos, D.J. Kazierad, J.R. Koup, A.J. Sedman, J. Clin. Pharmacol. 37 (12) (1997) 1114–1120.

[4] A. Shibukawa, T. Sawada, C. Nakao, T. Izumi, T. Nakagawa, J. Chromatogr. A 697 (1–2) (1995) 337–343.