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Journal of Pharmaceutical and Biomedical Analysis
29 (2002) 593–607

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

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Determination of pioglitazone hydrochloride in bulk and pharmaceutical formulations by HPLC and MEKC methods

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Received 29 August 2001; received in revised form 19 November 2001; accepted 1 December 2001

Abstract

High Performance Liquid Chromatographic (HPLC) and Micellar Electrokinetic Chromatographic (MEKC) methods have been developed for the determination of pioglitazone, a new englycemic antidiabetic agent. Pioglitazone and its unsaturated impurity were separated by MEKC in less than 7 min using a 43 cm × 50 μm i.d. uncoated fused-silica capillary with extended light path for better sensitivity (25 kV at 30 °C) and a background electrolyte (BGE) consisting of 20% acetonitrile (v/v) in 20 mM sodium borate buffer pH 9.3 containing 50 mM sodium dodecyl sulphate (SDS). The influence of various parameters on the separation such as pH of the buffer, SDS concentration, buffer concentration, organic modifiers, temperature and voltage were investigated. The MEKC method was compared with HPLC method using a 5 μm symmetry *C*18 column (250 × 4.6 mm i.d.) eluted with a mobile phase consisting of a mixture of 50% (v/v) acetonitrile and 10 mM potassium dihydrogen phosphate buffer, adjusting the pH to 6.0 with 0.1 M KOH. The HPLC method is capable of detecting all process related compounds, which may be present at trace levels in finished products. Both methods were fully validated and a comparison was made. The results confirm that the methods are highly suitable for its intended purpose.

Keywords: Pioglitazone; Process intermediates; Assay; Formulation; Validation and comparison

1. Introduction

Pioglitazone hydrochloride, (±)-5-{[[4-2-(5-ethyl-2-pyridinyl)ethoxy]phenyl]methyl}-2,4-thiazolidinedione monohydrochloride (Fig. 1), is an oral anti-hyperglycemic agent which acts primarily by decreasing insulin resistance and was developed by Takeda chemicals. It is used in the treatment of type-II diabetes (non-insulin-dependent diabetes mellitus, NIDDM also known as adult-onset diabetes).

So far very few liquid chromatography (LC) procedures have been reported for the determination of pioglitazone and its metabolites in biological fluids [1–3]. However, there are no other publications concerning the analysis of pioglitazone in bulk drug and in pharmaceutical dosage forms by LC.

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0731-7085/02/$ - see front matter
PII: S0731-7085(02)00036-5

Capillary electrophoresis (CE) has gained greatly in popularity during the last few years and is now routinely applied in pharmaceutical laboratories [4–11]. CE offers a broad range of selectivity in combination with high separation efficiency, working with minute sample volume, short analysis time and is therefore truly an alternative and complementary technique to High Performance Liquid Chromatographic (HPLC). Despite their apparent differences, combinations of CE and HPLC are frequently employed in cross-validation studies during method validation. The choice between adopting CE or HPLC for a particular application is dependent upon the relative merits of each method. Hitherto, the CE method for the analysis of pioglitazone hydrochloride is not reported.

The objective of this work was to develop analytical LC and CE procedures, which would serve as reliable and rapid methods for the determination of pioglitazone in bulk drug and in pharmaceutical formulations.

In the developed LC method, all the process intermediates were well separated and eluted before 20 min run time. It is likely that the unreacted intermediates and their precursors may remain as impurities in the final drug substance and affect its quality. Therefore, separation and determination of pioglitazone and its process related impurities/intermediates is important not only for quality assurance but also for process development. Hence the method is very useful for process monitoring during the production of pioglitazone. This particular approach has been found to be useful to control the level of impurities in the finished products. 4-Chloroaniline was

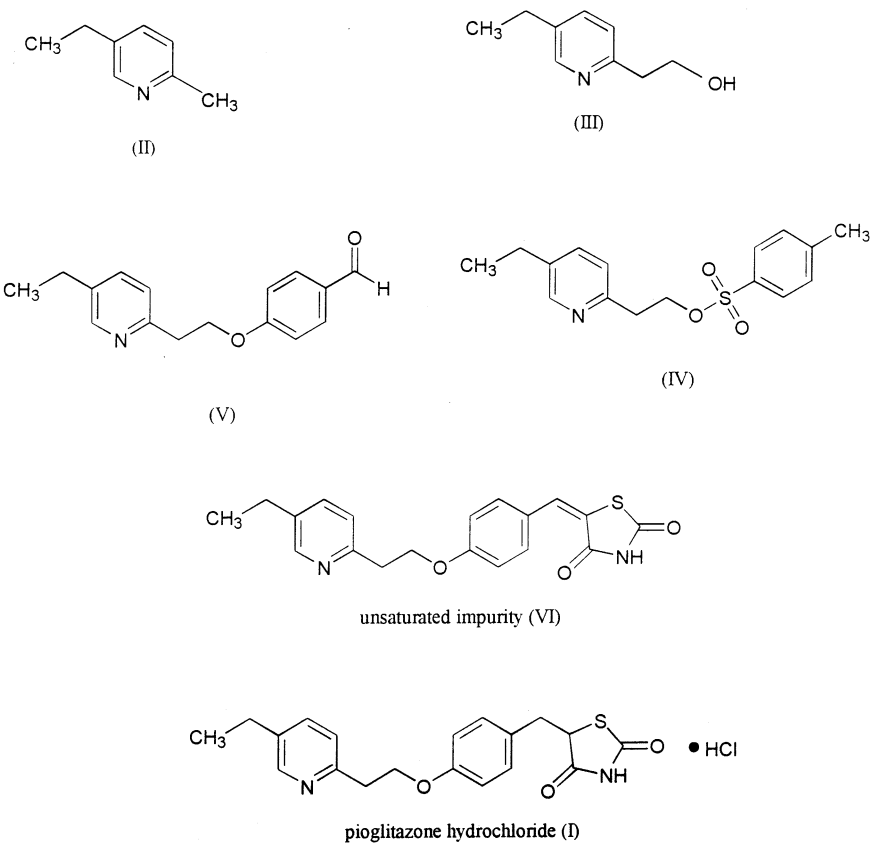

Fig. 1. Structures of pioglitazone hydrochloride and its related compounds.

used as internal standard for the purpose of quantitation.

The described Micellar Electrokinetic Chromatographic (MEKC) method is capable of separating pioglitazone and its unsaturated impurity (Fig. 1) in less than 7 min using a 43 cm × 50 μm i.d. uncoated fused-silica capillary with extended light path (25 kV at 30 °C). The background electrolyte (BGE) consisting of 20% acetonitrile (v/v) in 20 mM sodium borate buffer pH 9.3 containing 50 mM sodium dodecyl sulphate (SDS). The influence of various parameters on the separation such as pH of the buffer, SDS concentration, buffer concentration, organic modifiers, temperature and voltage were investigated. 4-Nitrophenol was used as internal standard for the purpose of quantitation of pioglitazone.

The developed LC and MEKC methods were subsequently validated. Both described methods are capable of quantitative estimation of pioglitazone in pharmaceutical dosage forms. The assay results obtained by MEKC are in fair agreement with those obtained by HPLC. The analysis of variance showed no significant difference between the results obtained by the two methods.

2. Experimental

2.1. Materials

Samples of pioglitazone and its process intermediates were received from Process R&D division of Dr Reddy's Research Foundation, Hyderabad, India. HPLC grade acetonitrile was obtained from Merck, USA. Analytical grade potassium dihydrogen phosphate, *ortho*-phosphoric acid and potassium hydroxides were purchased from Qualigens. Boric acid was obtained from Aldrich Chemical Company, USA. Analar grade sodium hydroxide was procured from BDH, E-Merck (India) Limited, India. SDS was purchased from Fluka, Switzerland. Tablets of Actos were purchased from the market. Each Actos tablet consists of 45 mg of API and the rest are excipients. High purity water was prepared by using Waters Milli-Q plus purification system.

2.2. Instrumentation

2.2.1. High performance LC

The LC system consisted of a Waters 510 pump with 996 Photodiode array detector and a Rheodyne injector equipped with 10 μl sample loop was used for the chromatographic separations in the parent lab (laboratory A). Waters LC Module I Plus was used for ruggedness testing in laboratory B. The output signals were monitored and integrated using Millenium 2010 Chromatography Manager software (Waters) in both the laboratories.

2.2.1.1. Chromatographic separation conditions. The mobile phase consisted of a mixture of aqueous 10 mM potassium dihydrogen phosphate–acetonitrile (50:50, v/v) adjusted to a pH of 6.0 with 0.1 M potassium hydroxide. The mobile phase was filtered through a 0.45-μm nylon membrane filter prior to use. Symmetry C18 analytical column (250 × 4.6 mm, 5 μm packing) (Waters) was used for the separation. The flow rate of mobile phase was 1.0 ml/min and the column was operated at ambient temperature (~ 22 °C). The sample injection volume was 10 μl. The photodiode array detector was set at a wavelength of 225 nm.

2.2.1.2. Diluting solution. A mixture of aqueous 0.1% *ortho*-phosphoric acid and acetonitrile in the ratio of 1:1 (v/v).

2.2.1.3. Preparation of samples. Sample solutions of pioglitazone and process impurities were prepared with diluting solution. The working analyte concentration of pioglitazone for assay determination and for related substance determination was 0.5 mg/ml. The concentration of the internal standard was maintained at 400 μg/ml in each solution of pioglitazone and was used for validation studies.

2.2.1.4. Degraded samples for specificity study. For acid degradation, pioglitazone sample was refluxed with 0.1 N HCl at 60 ± 1 °C for 12 h and then neutralized by adjusting pH to 7.0 with 0.1 N NaOH. The solution was further diluted to the required concentration with the mobile phase.

For basic degradation, pioglitazone sample was refluxed with 0.1 N NaOH at 60 ± 1 °C for 12 h and then neutralized by adjusting pH to 7.0 with 0.1 N HCl. The solution was further diluted to the required concentration with mobile phase.

For oxidative degradation, pioglitazone sample was refluxed with 3% H_2O_2 for 3 h and then diluted to the required concentration with mobile phase.

For photo and thermal degradations, two separate solutions of pioglitazone (0.5 mg/ml in diluting solution) were prepared. One solution was exposed to ultraviolet light (254 nm) for 24 h, and the other was kept at 50 °C for 12 h.

2.2.1.5. Preparation of sample from formulation. Twenty weighed tablets of Actos (45 mg of pioglitazone) were ground to a fine powder. The amount of powder equivalent to 50 mg of the active compound was extracted with diluting solution and centrifuged. The supernatant was taken as stock solution.

2.2.2. Capillary electrophoresis

An Agilent Technologies CE (Model G1600 AX) system equipped with an on-column diode array detector and an autosampler. A 3D-CE Chemstation software Rev. A. 08.01(783) (Agilent Technologies) was used for instrument control, data acquisition and data handling. The extended light path fused-silica capillary (Hewlett Packard, Wardbronn, Germany) of 43 cm long (34.5-cm effective length) with a 50 μm i.d. was used. The pH adjustments of buffers were made with Metrohm autotitrator using Metrohm 6.0222.100 pH electrode.

2.2.2.1. Preparation of BGE. Twenty millimolar sodium borate buffer was prepared by dissolving 123.6 mg of boric acid in about 80 ml and adjusting the pH with 0.1 N sodium hydroxide to a pH of 9.3 and then make up the volume to 100 ml. Then required amounts of SDS were weighed and dissolved in the sodium borate buffer to maintain the concentration of 50 mM. The buffer was filtered through 0.45 μm Nylon-66 filter using vacuum pump. Finally, BGE was prepared by mixing 80 parts of 20 mM sodium borate pH 9.3 buffer containing 50 mM SDS and 20 parts of acetonitrile.

2.2.2.2. Capillary preparation and precondition. New bare fused-silica capillaries were flushed with 1 M NaOH for 20 min followed by HPCE water for 10 min, 0.1 M NaOH for 20 min, HPCE water for 10 min and running buffer by 20 min. Precondition of the capillary for the BGE was programmed for each run and consisted of 2 min flush with 0.1 M NaOH and followed by 3 min flush with run buffer.

Prior to each sequence of samples, two blank injections were performed for stabilizing the capillary wall surface, and allowing the buffer and sample solutions to reach a constant temperature on the auto-sampler tray. Finally, triplicate injections were performed.

2.2.2.3. Electrophoretic separation conditions. BGE contains 80 parts of 20 mM sodium borate (pH 9.3) containing 50 mM SDS and 20 parts of acetonitrile. A diode array UV detector was set at 210 nm with a bandwidth of 4 nm. The temperature maintained constant throughout at 25 °C. A constant voltage of 25 kV, with an initial ramping of 1000 V/s, was applied during analysis. Hydrodynamic sample injection was performed at 50 mbar over 3 s.

2.2.2.4. Diluting solution. A mixture of water–acetonitrile in the ratio (80:20).

2.2.2.5. Solutions. All stock solutions were prepared first using running buffer and then the sample solutions of required concentrations prepared from stock by diluting them using diluent to favor sample stacking, and were introduced to the capillary by pressure injection. The working analyte concentration of pioglitazone for assay determination was 75 μg/ml and for related substance determination was 350 μg/ml. The concentration of the internal standard (4-nitrophenol) was maintained at 500 μg/ml in each solution of pioglitazone and was used for validation studies. All samples were filtered through a 0.45 μm filter before use.

2.2.2.6. Formulated sample. Twenty weighed tablets of Actos (equivalent to 45 mg of pioglitazone in each tablet) were ground to a fine powder. The amount of powder equivalent to 50 mg of the active compound was extracted with running buffer and centrifuged. The supernatant was diluted with diluent to prepare samples of required concentrations.

3. Results and discussion

3.1. High performance liquid chromatographic method

3.1.1. Separation of process impurities

Pioglitazone and all its process related compounds of (**II**, **III**, **IV**, **V** and **VI**) are shown in Fig. 1. The chromatographic conditions to be optimized to obtain the best separation between all these compounds are shown in Table 1. To develop a suitable and robust LC method for the determination of pioglitazone and its intermediates, different mobile phases and columns were employed to achieve the best separation and resolution. The method development was initiated with a C18 column using a mobile phase of water–acetonitrile in the ratio of 1:1. In the above conditions elution was very broad for pioglitazone and compound **VI**. Introduction of 10 mM potassium dihydrogen phosphate buffer in the above mobile phase gave sharp peaks, but poor separation was observed for pioglitazone and compound **IV**. Early elution with a little separation was observed for all compounds with the mobile phase consisting of aqueous 10 mM potassium dihydrogen phosphate–acetonitrile (50:50, v/v), pH adjusted to 3.5. Interestingly, the same mobile phase with pH 7.0, gave reasonable separation between all compounds. In the above conditions compound **VI** eluted prior to pioglitazone, but the separation was not up to base line and therefore needs further optimization. Finally, the mobile phase consisting of aqueous 10 mM potassium dihydrogen phosphate–acetonitrile (50:50, v/v) mixture adjusted to pH 6.0 was found to be appropriate allowing good separation of all the compounds at a flow rate of 1.0 ml/min using symmetry *C*18, 250 mm column. The chromatogram of crude pioglitazone sample spiked with all the related substances recorded using the proposed method is shown in Fig. 2.

In the presented method for all the compounds the selectivity was found to be more than 1.0 with a resolution more than 2.4. System suitability results of the method are presented in Table 1. Pioglitazone and its related compounds show significant UV absorbance at wavelength 225 nm. Hence this wavelength has been chosen for detection in the analysis of pioglitazone.

3.1.2. Quantification of unsaturated impurity

For the quantification of impurities, a high–low chromatographic technique [12] was used. In this technique a concentrated (3.5 mg/ml) sample of pioglitazone was injected and the response of each impurity was recorded. A typical chromatogram of high–low chromatography of pioglitazone is shown in Fig. 3. The impurities can be identified by matching the UV spectrum and the retention times with that of standards. Weight percentages of each impurity present in pioglitazone sample (in 3.5 mg/ml) were calculated using its peak response and relative response factors (RRFs). The RRF of unsaturated impurity with respect to pioglitazone was found to be 0.69 at the detection wavelength of 225 nm. RRF is the ratio of the peak response per unit concentration for each impurity to the peak response per unit concentration for the reference compound (pioglitazone) under the given analytical conditions. The impurities namely UK1, UK2, and UK3 eluted at retention times 2.97, 4.18 and 4.68 min, respectively, were not characterized (Fig. 3). UV absorption spectra of these impurities are shown in the same figure. Using RRF value and its peak response, the weight percentage of the unsaturated impurity present can be calculated [13].

3.1.3. Validation

The described LC method has been extensively validated for assay and unsaturated impurity of pioglitazone using the following parameters. 4-Chloroaniline was used as internal standard for the purpose of quantification of pioglitazone.

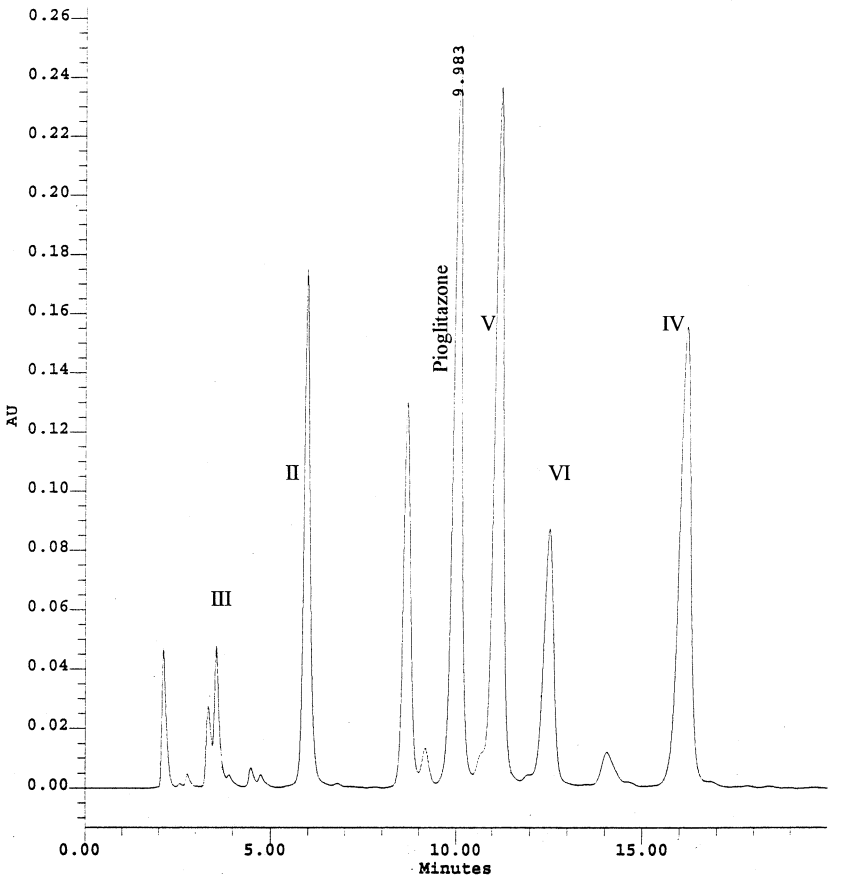

Fig. 2. HPLC chromatogram of pioglitazone and its process intermediates.

Table 1
System-suitability report of HPLC method

Compound	Capacity factor[a]	Selectivity[a]	Tailing factor[a]	Resolution[a]	Theoretical plates[a]
Pioglitazone	3.6	2.07	1.15	12.28	12 853
II	1.74	3.22	1.22	8.5	7361
III	0.54	–	0.982	–	4548
IV	6.4	1.32	1.13	5.82	13 999
V	4.06	1.13	1.11	2.43	11 119
VI	4.85	1.19	1.05	3.64	10 335

[a] Number of samples analyzed is three.

3.1.3.1. Specificity. Complete resolution of pioglitazone from its related compounds, with good peak symmetry and no apparent shoulders, is already shown in Fig. 2.

To demonstrate the specificity of the method, all the possible known impurities discussed above were added to pure pioglitazone sample and the mixture was analyzed for assay and the results were compared with pure sample results. Reproducibility was observed in both the cases (RSD < 2.0)

Accelerated degradation studies were also performed to demonstrate the validity of the method. The samples, which are refluxed with 0.1 N HCl or subjected to high temperature or exposed to UV light, did not give any degradation products. But the sample refluxed with 0.1 NaOH, completely decomposed to give several degraded products. Fig. 4 shows the separation of the pioglitazone and their base catalyzed hydrolysis products. With oxidative degradation, pioglitazone mostly converted to an oxidative degradation product (eluted at 2.2 min), which was not characterized.

Photodiode array detection was used as evidence of the specificity of the method, and to evaluate the homogeneity of the peak. Chromatographic peak purity was determined using wavelength comparison (225 vs 265 nm) [14]. The samples exposed to acidic, basic, oxidative, thermal and UV stress conditions were subjected to photo-diode array analysis for peak purity of pioglitazone. The plots with flat tops in all instances showed that pioglitazone peak had no detectable impurity peaks embedded in and are free of co-eluting degradation compounds. From the above results, it is clear that the method can

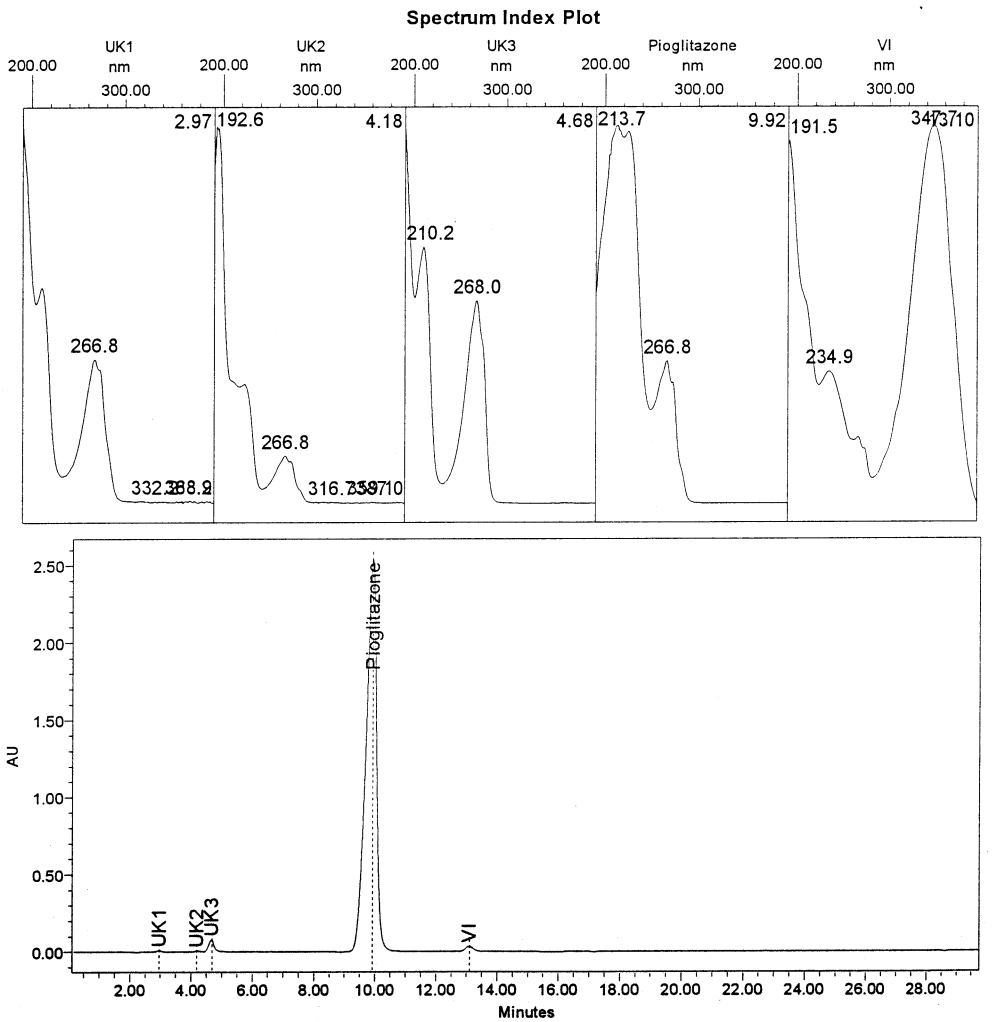

Fig. 3. High–low chromatogram of pioglitazone hydrochloride with spectrum index plot for all components using PDA detection.

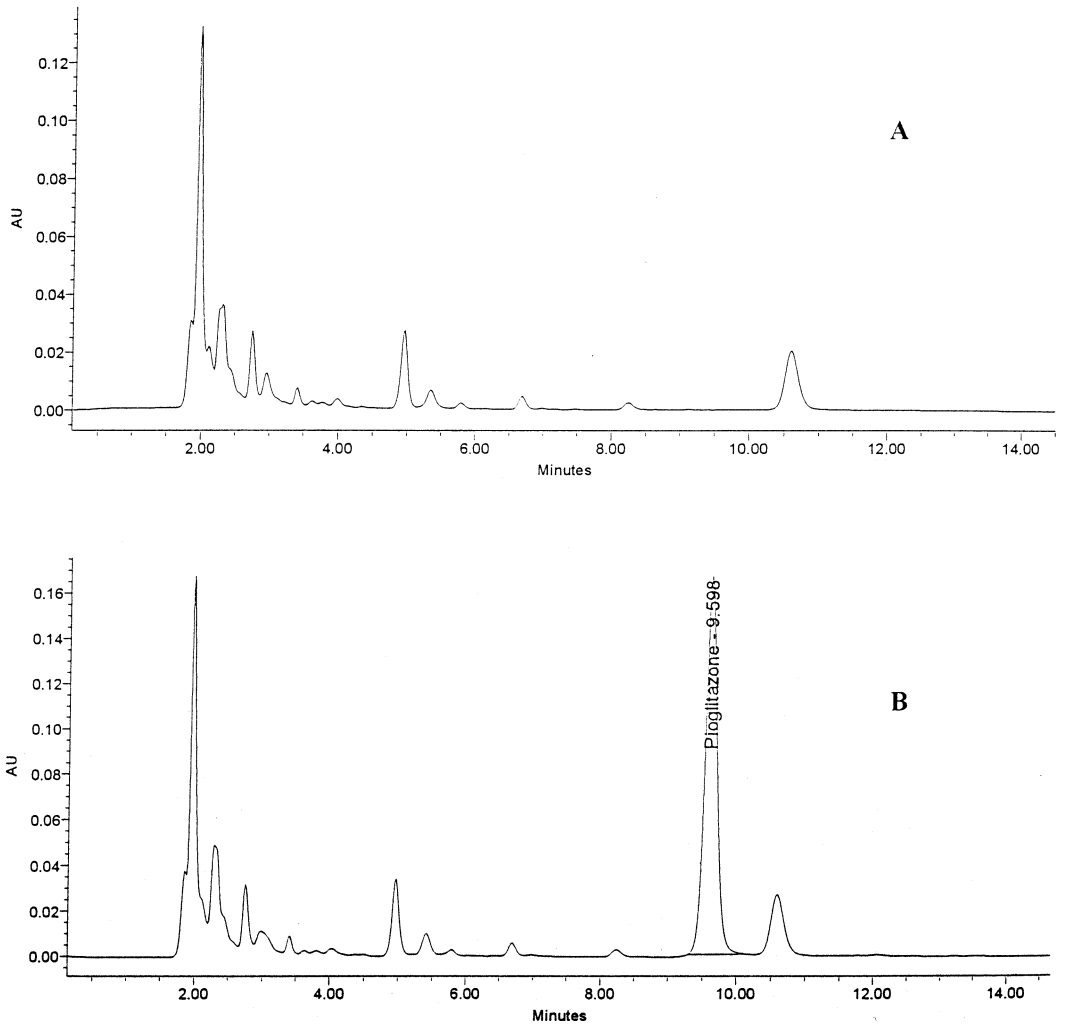

Fig. 4. (A) Chromatogram of base degraded products of pioglitazone sample. (B) Separation of pioglitazone from its base degradation products.

be used for determining the stability of pioglitazone as bulk and pharmaceutical formulations.

3.1.3.2. Linearity. Linearity was checked by preparing standard solutions at seven different concentration levels ranging from 135 to 685 μg/ml. The linearity was also checked for 3 consecutive days for the solutions of the same concentrations prepared from the stock solution. The precision for inter-day linearity is below 1.62% RSD. The equation for calibration curve is $y = 1.87x - 0.019$. The correlation coefficient was found to be more than 0.999, indicating good linearity.

3.1.3.3. Accuracy. The accuracy of the method for assay determination was checked at three concentration levels i.e. at 200, 300, and 400 μg each in triplicate for 3 consecutive days. Solutions for the standard curves were prepared fresh every day. The assay accuracy variation shown in terms of relative mean error (RME), total error (TE) and % recovery are tabulated in Table 2 [15]. The RME and TE values are below ± 2.0 for the intra-day assay experiments.

Table 2
Accuracy in the assay determination of pioglitazone by HPLC

Day of analysis	Taken (mg)	Recovery (mg) ($n = 3$)	% Recovery	RME (%)	TE (%)
0 day	0.205	0.204	99.51	−0.68	−0.283
	0.314	0.312	99.36	−0.64	−0.24
	0.408	0.409	100.24	0.163	0.469
1 day	0.205	0.205	99.80	−0.163	0.143
	0.314	0.314	99.90	−0.106	0.20
	0.408	0.411	100.73	0.65	0.956
2 days	0.205	0.206	100.48	0.49	0.89
	0.314	0.315	100.32	0.43	0.66
	0.408	0.411	100.73	0.82	1.05

Standard addition and recovery experiments were also conducted to determine the accuracy of the present method for the quantification of unsaturated impurity. The range of addition levels of impurity to the parent compound was done at 0.2–1.0% of the concentration (0.5 mg/ml) of pioglitazone. The recovery of impurity was calculated from the slope and intercept of the calibration graph drawn in the concentration range 0.25–10 μg (0.05–2%) using authentic standard [16]. The equation for calibration curve is $y = 21\,013x + 644$ with a correlation coefficient more than 0.991. The intercept value was found to be less than the 10% of area response produced by 2% concentration level [17]. The mean recovery of impurity was found to be in the range of 95–104.5%.

3.1.3.4. Precision. The precision of the method for the determination of assay and unsaturated impurity of pioglitazone was studied using the parameters viz. repeatability, intermediate precision and reproducibility (Ruggedness).

Repeatability in the intra-day variations in assay or in recovery of unsaturated impurity obtained at different concentration levels is expressed in terms of RSD values calculated from the data of each day for 3 days. RSD values of assay and recovery of impurity were found to be below 1.0 (Table 3) and 2.7%, respectively.

The intermediate precision, which is the inter-day variation at the same concentration level, was determined on successive days. The intermediate precision for assay and recovery of impurity of pioglitazone were found to be 0.5 (Table 3) and 3.5% RSD, respectively.

The ruggedness of an assay method is defined as the degree of reproducibility of the results obtained by analysis of the same sample under a variety of normal test conditions such as different labs, analysts, instruments and lots of reagents. The samples of day 2 (parent lab) were analyzed at laboratory B with a different instrument (LC

Table 3
Inter- and intra-days assay variation of pioglitazone using HPLC method

Intra-day			
0 day			
Mean of concentration (mg/ml) $n = 3$	0.204	0.312	0.409
SD	0.002	0.002	0.0015
RSD (%)	0.980	0.641	0.374
1 day			
Mean of concentration (mg/ml) $n = 3$	0.205	0.314	0.411
SD	0.0015	0.0015	0.0015
RSD (%)	0.746	0.487	0.372
2 day			
Mean of concentration (mg/ml) $n = 3$	0.206	0.315	0.411
SD	0.002	0.0012	0.0012
RSD (%)	0.970	0.366	0.280
Inter-day			
Mean	0.205	0.314	0.410
SD	0.0010	0.0015	0.0013
RSD (%)	0.50	0.48	0.32

Table 4
Assay values of pioglitazone from Actos tablets using HPLC

Amount taken (mg) ($n = 3$)	Amount recovered (mg)	% Recovery
0.215	0.213	99.23
0.322	0.327	101.56
0.430	0.425	98.93
0.537	0.543	101.22
0.645	0.650	100.77

Module I plus HPLC system) by a different analyst with different lots of reagents and another batch column. The data obtained from laboratory B were within 2% RSD when compared with the data of the parent lab.

3.1.3.5. Limits of detection (LOD) and quantification (LOQ). The detection sensitivity can be demonstrated by the LOD. A signal-to-noise ratio of approximately 2–3 is generally considered to be acceptable for estimating the detection limit, which is the lowest concentration that can be detected. The LOQ is the lowest concentration of a substance that can be quantified with acceptable precision and accuracy. A typical signal-to-noise ratio is 10:1. The LOD and LOQ values for unsaturated impurity were found to be 0.08 and 0.25 μg, respectively.

3.1.4. Assay determination of pioglitazone from Actos tablets

The supernatant of tablets extract was diluted with diluting solution to prepare five independent samples of different concentrations ranging from 0.2 to 0.6 mg/ml. The assay results of the extracted samples were comparable with claimed values (Table 4). Pioglitazone and the internal standard were well separated from the excipient peaks in the formulation sample. A typical LC chromatogram is shown in Fig. 5.

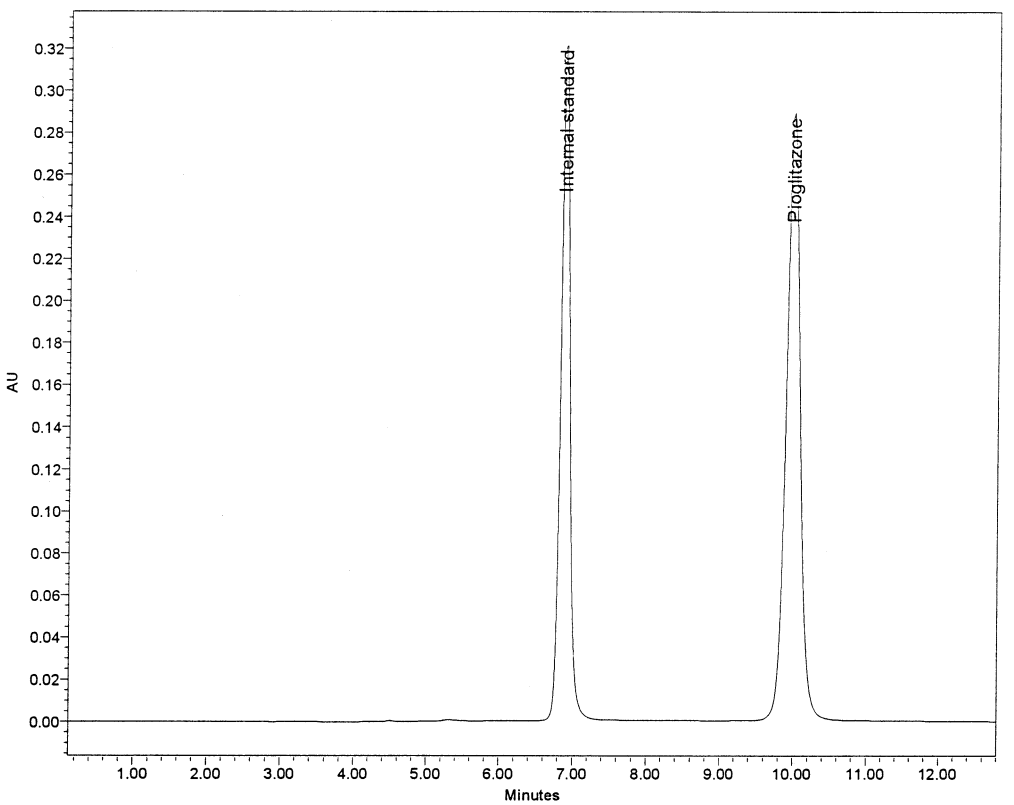

Fig. 5. Chromatogram of formulated pioglitazone (Actos 45 mg) with internal standard.

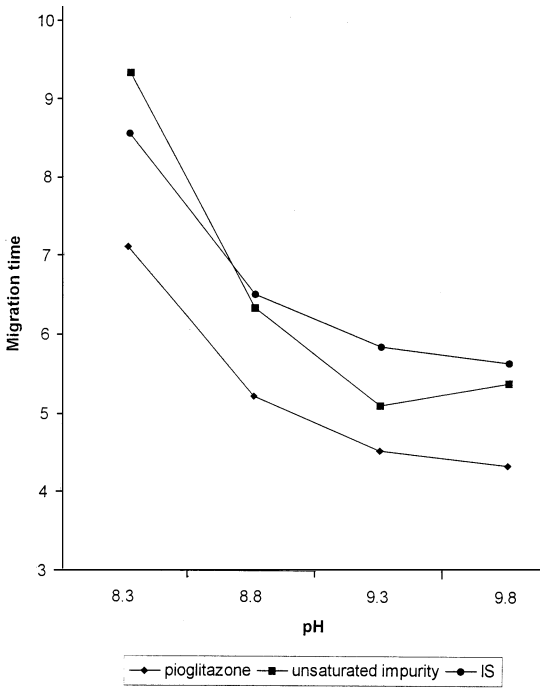

Fig. 6. Influence of pH on migration of the compounds.

3.2. Capillary electrophoresis

3.2.1. Method optimization

In order to develop a CE method for the separation of pioglitazone and its unsaturated impurity, different buffers at different pH values and concentrations were tested using capillary zone electrophoresis (CZE). None of the attempts gave satisfactory separation in CZE mode. Then MEKC mode was selected for the separation. The method development was started with a running buffer consisting of 40 mM SDS in a mixture of 20 mM buffer–acetonitrile in the ratio 8:2, 25 kV applied voltage and a temperature of 30 °C. Owing to the poor solubility of pioglitazone and its impurity in the aqueous buffers, acetonitrile was introduced in the running buffer. The influence of following parameters on the separation was investigated.

The buffer pH is one of the most important parameters for improving selectivity in CE, especially for closely related compounds. The pK_a value of pioglitazone was between 5.8 and 6.4 in methanol at 25 °C. Hence the pH range of 7–10 was selected for the study. The migration of compounds under study was decreased with decrease of pH and not even eluted at BGE pH 7.0 up to 30 min. Finally, sodium borate buffer pH at 9.3 was selected for further experiments since it gave good resolution, peak shapes and short run time for analysis. The influence of pH on migration of pioglitazone, unsaturated impurity and internal standard is shown in Fig. 6.

The molarity of the borate buffer was varied from 10 to 40 mM using the experimental conditions mentioned above and its effect on separation was studied. An increase in the buffer concentration resulted in a decrease in the electroosmotic flow (EOF) due to compression of the double layer [18] and thereby an increase in migration times of the analytes. The two compounds were well separated in the buffer range studied. However, 20 mM concentration was considered as suitable for its good resolution, peak shape and run time.

An SDS concentration range from 35 to 65 mM was taken for this part of the study keeping the borate concentration at 20 mM and pH at 9.3. The results show that an increase of SDS concentration has influence on the retention of the compounds studied. It can be seen that as the number of micelles increased, the concentration of solute in the micelles is increased which results in its low mobility. A 50-mM SDS concentration was selected for further experiments since it gave good resolution and high narrow peaks making it easier for integration.

The effect of concentration of acetonitrile on the separation was explored and the concentrations were varied from 18 to 22%. The resolution between pioglitazone and unsaturated impurity decreased when the concentration of acetonitrile increased. However, 20% of acetonitrile was selected for the separation that provided a compromise between sufficient resolution and runtime.

The effect of the temperature on the separation was tested between 20 and 40 °C. The resolution between pioglitazone and unsaturated impurity decreased when the temperature was increased. According to resolution, runtime, and current generated (50 μA), a temperature of 30 °C was selected as suitable.

The effect of varying the voltage from 15 to 30 kV was investigated under the conditions set out above. The resolution between pioglitazone and unsaturated impurity decreased with increase in voltage. A potential of 25 kV yielded the best compromise in terms of run time, current generated and linearity between voltage and current.

Finally, the optimized conditions for the separation and quantification of pioglitazone and its impurity were; BGE: 80 parts of 20 mM sodium borate buffer (pH 9.3) containing 50 mM SDS and 20 parts of acetonitrile, 25 kV voltage and 30 °C temperature. Under these conditions, pioglitazone can be completely separated from its unsaturated impurity below 7 min run time.

4-Nitrophenol was used as internal standard for the purpose of quantitation of pioglitazone. Separation of pioglitazone with its impurity and internal standard using the selected conditions is shown in Fig. 7. System-suitability results of the developed method are given in Table 5.

3.2.2. Method validation

A validation study was performed to demonstrate the developed method was practical. The validation was carried out in a similar way to that generally adopted for HPLC. The developed CE method was validated for the determination of pioglitazone and its unsaturated impurity in bulk drug and formulation samples.

3.2.2.1. Specificity. The electropherogram of real analyzed sample in Fig. 8 shows the separation of pioglitazone from its unsaturated impurity. All

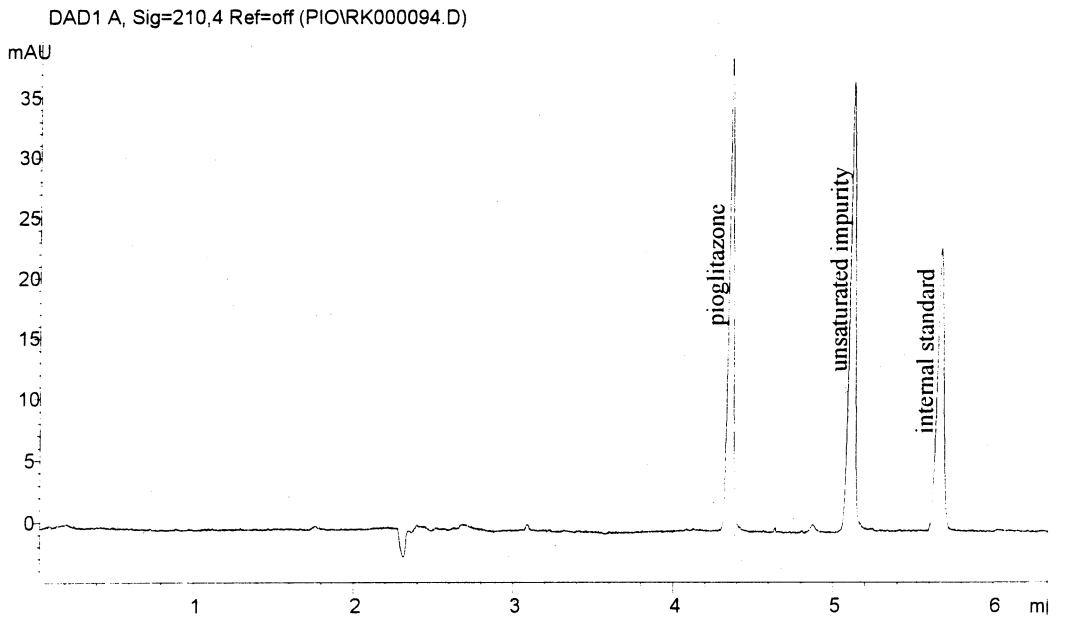

Fig. 7. Electropherogram of pioglitazone separated from its unsaturated impurity and internal standard.

Table 5
System-suitability results of MEKC method

Compound	Migration times	Selectivity	Symmetry factor	Resolution	Theoretical plates
Pioglitazone	4.44	–	1.28	–	156 873
Unsaturated impurity	5.31	1.20	1.18	17.20	140 841
Internal standard	5.68	1.07	1.20	5.82	105 202

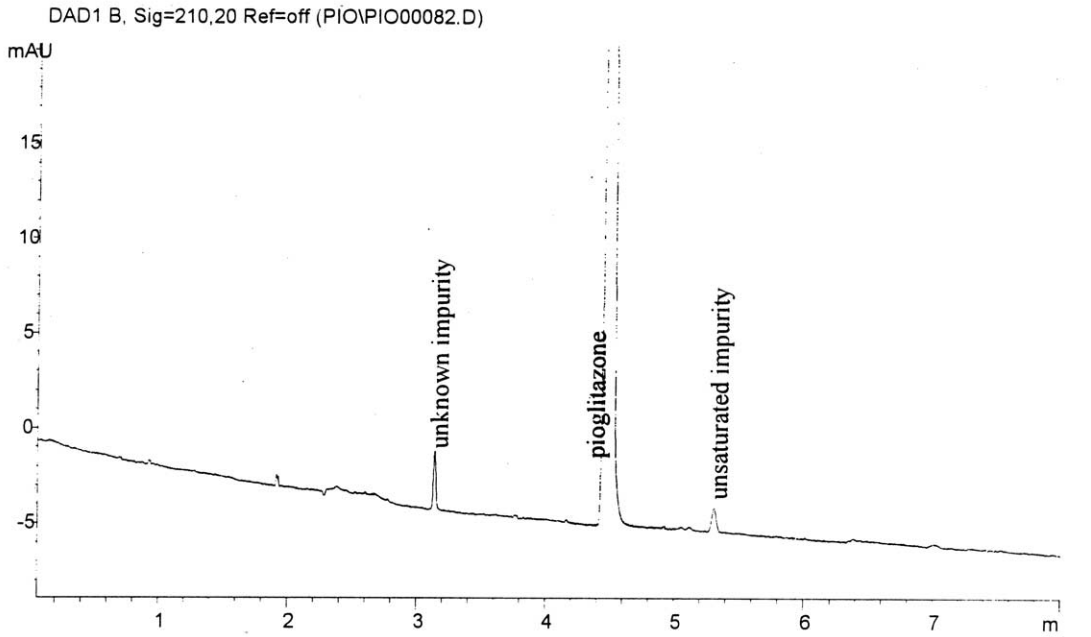

Fig. 8. Electropherogram of real analyzed sample of pioglitazone.

components are baseline separated at relevant concentration levels. Peak purity test for pioglitazone indicated the absence of co-eluting components.

3.2.2.2. Precision. Method precision was determined by measuring repeatability and intermediate precision (inter-day precision) of migration times, peak area, peak area ratio, and % peak area for pioglitazone and its impurity.

In order to determine the repeatability of the method, replicate injections ($n = 6$) of a 0.5% of unsaturated impurity spiked in pure pioglitazone (0.5 mg/ml) were injected. The relative standard deviation (RSD) for the above parameters of each compound are given in Table 6.

Three sample preparations ($n = 3$) of the same sample were analyzed on three different days to explore intermediate precision. The results are tabulated in Table 6.

3.2.2.3. Linearity. Linearity was checked by preparing standard solutions at five different concentration levels of pioglitazone and impurity in the range from 20 to 100 μg/ml and 0.1–2.5% (0.35–8.75 μg/ml), respectively. Standard solutions of linearity of impurity were prepared by spiking the impurity with pure pioglitazone, which is at a concentration of 0.35 mg/ml. The linear regression lines were calculated by the method of least-squares. The equations for calibration curve of both pioglitazone and unsaturated impurity are $y = 1.393x + 0.0426$ and $y = 0.876x + 0.217$, re-

Table 6
Precision of MEKC method

	Pioglitazone (RSD %)	Impurity (RSD %)
Repeatability		
Migration time	1.77	1.82
% Peak area	0.21	5.57
Peak area	2.89	7.04
Peak area ratio	1.71	–
Intermediate precision		
Migration time	2.12	2.35
% Peak area	0.24	6.29
Peak area	3.26	8.25
Peak area ratio	1.94	–

Table 7
Recovery studies by MEKC

Concentration added (μg/ml)	Concentration found (μg/ml)[a]	Recovery (%)
Pioglitazone		
50	50.2 ± 0.72	–
+25	75.6 ± 0.78	101.4
+45	95.6 ± 0.92	100.9
+65	116.2 ± 0.80	101.5
Unsaturated impurity		
1.52	1.59 ± 0.079	104.6
3.04	3.13 ± 0.086	103.2
4.58	4.42 ± 0.18	96.5
6.08	5.85 ± 0.09	96.2

[a] Mean ± SD ($n = 3$).

spectively. The correlation coefficients were found to be more than 0.998 in both cases.

3.2.2.4. Accuracy. The accuracy of the method was tested by analyzing the solutions prepared from commercial formulation of pioglitazone (Actos tablets) to which three different known amounts of standards were added. The concentration of the solution being spiked was 50 μg/ml and the three spiking concentrations were 25, 45, and 65 μg/ml. The results listed in Table 7 show that the recoveries are within 100 ± 1.5%.

Standard addition and recovery experiments were also conducted to determine the accuracy of the MEKC method for the quantification of unsaturated impurity. The unsaturated impurity was spiked at three concentration levels ranging from 0.3 to 1.0% (w/w) in a formulation sample of pioglitazone at its target analyte concentration (0.35 mg/ml), each level was injected in triplicate. All the recovery data are given in Table 7. The % recoveries are ranging from 96.2 to 104.6.

3.2.2.5. Ruggedness. The methods were performed by different chemists with different capillary lots, different lots of reagents on different days. The changes of the migration times of all the compounds were within a ± 0.3-min window with all these variations.

3.2.2.6. Limit of detection and quantification. For the LOD and LOQ tests, a solution of unsaturated impurity (18.4 mg/25 ml) was diluted gradually. The solutions corresponding to 0.04 (0.29 μg/ml) and 0.1% (0.74 μg/ml) were found to correspond to the LOD and LOQ, respectively.

3.3. Comparison of assay results using MEKC and HPLC

The assay results of pioglitazone in pharmaceutical dosage forms obtained by both LC and MEKC methods are given in Table 8. The values shown in the table exemplify the high precision of the proposed methods. The results obtained from the MEKC method were compared statistically by Student's *t*-test and *F*-tests with the HPLC method and found that the MEKC method does not differ significantly in precision and accuracy from the HPLC method.

Table 8
Assay of pioglitazone in pharmaceutical formulation using MEKC and HPLC

Label amount (mg/ml)	Found by MEKC # (mg)	Found by HPLC # (mg)	*t*-test[a]	*F*-test[a]
45	44.82	44.65	0.134	2.48
	46.27	45.70		
	44.22	44.51		
	45.89	45.54		
	44.84	45.34		
Mean	45.21	45.15		
SD	0.844	0.536		

[a] Theoretical values at 95% confidence limit, $t = 2.31$, $F = 9.60$; # $n = 3$.

4. Conclusion

HPLC and MEKC methods have been developed and validated for the quantitative determination of pioglitazone and its impurity in bulk and in pharmaceutical dosage form. The developed HPLC method was found to be selective, sensitive, precise and stable indicating pioglitazone and its process related impurities. Also, kinetic measurements can be carried out by HPLC method to determine completion times for synthetic reactions. The MEKC method serves as an alternative method for the determination of API in commercial samples of pioglitazone. The assay results obtained by these two methods are in fair agreement. These methods can be used for the routine determination of pioglitazone in bulk and pharmaceutical formulations.

Acknowledgements

The authors wish to thank the management of Dr Reddy's group for supporting this work. We would also like to thank colleagues in separation science division of Analytical Research of Dr Reddy's Research Foundation for their co-operation and encouragement in carrying out this work.

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