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Development and validation of a high-resolution capillary electrophoresis method for multi-analysis of ragaglitazar and arginine in Active Pharmaceutical Ingredients and low-dose tablets

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Abstract

A selective, sensitive and robust capillary electrophoresis (CE) method has been developed and validated for multi analysis of ragaglitazar (also known as NNC 61-0029 or DRF 2725) and its counter ion arginine in Active Pharmaceutical Ingredients (API) and low-dose tablets (0.5, 1.0 and 2.0 mg). The method covers a total number of 12 analyses for the API and tablets: assay and identification of ragaglitazar and arginine, chiral purity of ragaglitazar and purity of ragaglitazar.

After a simple extraction of samples with acetonitrile and 0.01 M sodium hydroxide (10:90), separation was performed using a combination of two cyclodextrins; sulfobutylether-β-cyclodextrin (SB-β-CD) and dimethyl-β-cyclodextrin (DM-β-CD) in the electrolyte. The method showed good specificity and could separate and detect ragaglitazar, the distomer (the (+)-enantiomer) and arginine. The LOQ was found to be 0.10%, corresponding to 0.2 ng (0.5 μg/ml) for ragaglitazar and the distomer. Linearity was observed to be from 0.5 to 15 μg/ml (range 0.2–60 ng) and 400–600 μg/ml (range 1603–2404 ng) for ragaglitazar and 166–250 μg/ml (range 668–1000 ng) for arginine. The accuracy (as percent recovery) for ragaglitazar was found to be 101–106% (at 400–600 μg/ml), 101–125% (at 0.5–15 μg/ml) and for arginine 97–101% (at 166–250 μg/ml). The repeatability for the detection of peaks as R.S.D. was found to be as follows, ragaglitazar: 0.05%, distomer: 1.01%, largest single impurity: 5.84%, total impurities: 0.90% and arginine: 2.00%. The intermediate precision for the detection of peaks as R.S.D. was found to be as follows, ragaglitazar: 0.63%, distomer: 1.98%, largest single impurity: 5.22%, total impurities: 13.17% and arginine: 3.50.

Keywords: Capillary electrophoresis; Cyclodextrin; Validation; Ragaglitazar; Arginine

1. Introduction

The development of a new drug, and finding the best pharmaceutical formulation, requires a large number of analyses. These analyses are very time-consuming

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and expensive. It is therefor important that the analytical methods are developed and chosen with careful consideration to achieve high-quality information rapidly and at low cost.

Capillary electrophoresis (CE) is recognized as a good complementary and/or alternative to high-performance liquid chromatography (HPLC) for pharmaceutical and biological applications [1–6]. In most cases, HPLC will be used in preference, since the separations achieved are more robust and analysts feel comfortable with its application. However, in some cases, a CE method may show clear advantages combined with a high selectivity, in which case it may be chosen as the method of choice [7]. Recently, a general test chapter on CE has been added to both the US Pharmacopoeia [8] and European Pharmacopoeia [9].

Here a description of a simple and selective CE method using two cyclodextrins as chiral selectors is presented for the determination of ragaglitazar, the distomer of ragaglitazar (the (+)-enantiomer) and arginine (the counter ion of ragaglitazar). The method covers a total number of 12 analyses for the Active Pharmaceutical Ingredients (API) and low-dose tablets (0.5, 1.0 and 2.0 mg) as assay and identification of ragaglitazar and arginine, chiral purity and purity of ragaglitazar.

Ragaglitazar belongs to a new class of dual acting PPARα and γ agonists, which restores insulin sensitivity and corrects diabetic dyslipidaemia in clinical trials. The drug is in development for the treatment of type 2 diabetes. The biological effect of ragaglitazar is exerted via two isoforms of the peroxisome proliferator activated receptor (PPAR) family, namely PPARγ and PPARα: Ragaglitazar; ((−) 3[4-[2-(phenoxazin-10-yl) ethoxy] phenyl]-2-ethoxy propanoic acid), $C_{25}H_{25}NO_5$, and its counter ion arginine are shown in Fig. 1.

To be able to separate and quantify the compounds in samples (API, 0.5 mg, 1.0 mg and 2.0 mg tablets) for multi determination analysis during development, a selective, sensitive and robust capillary electrophoresis (CE) method with UV detection was developed and validated. A simplified extraction procedure with good recovery for all tested compounds was developed and used for sample preparation. As part of the documentation, the method was validated according to official guidelines [10,11].

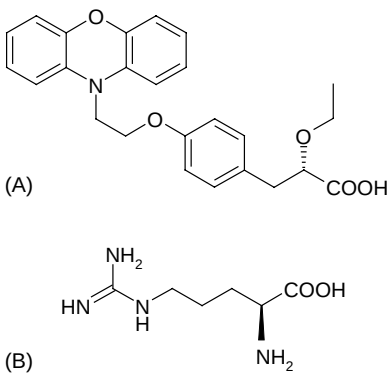

Fig. 1. The chemical structure of (A) Ragaglitazar a new class of dual acting PPARα and -γ agonist for treatment of type 2 diabetes and (B) Arginine, a counter ion of ragaglitazar.

2. Experimental

2.1. Chemicals, materials, reagents and solutions

Deionized water was obtained from a Milli-Q system, acetonitrile (ACN), HPLC grade (e.g. Rathburn), 0.01 M and 0.1 M sodium hydroxide, NaOH (e.g. diluted sodium hydroxide 1.0 M, Cat. No. LAB00330, from Bie & Berntsen, Copenhagen, Denmark), Sulfobutylether-β-cyclodextrine (e.g. Advasep, CyDex (SB-β-CD)), Dimethyl-β-cyclodextrine (e.g. Cyclolab, Hungary, 98% purity (DM-β-CD)), Sodium dihydrogen phosphate-monohydrate (Merck), ragaglitazar standard, arginine standard, ragaglitazar-related substances as standards, API and tablets (R&D Novo Nordisk A/S, Maaloev, Denmark), Arginine USP standard.

2.1.1. Preparation of solutions

Buffer (25 mM phosphate buffer pH 8.0 for manufacture of buffer electrolyte): In a 1000 ml measuring flask, 3.45 g sodium dihydrogen phosphate-monohydrate was dissolved in 900 ml water. The pH value was adjusted to 8.0 with sodium hydroxide (e.g. 40%) and/or phosphoric acid (exact adjustment e.g. 85%) and filled to the mark with water.

Buffer-electrolyte (for manufacturing of electrolyte): The buffer-electrolyte consisted two of cyclodextrins (2.0% SB-β-CD/0.7% DM-β-CD (w/w)) dissolved in 25 mM phosphate buffer pH 8.0. Preparation: 2.0% SB-β-CD + 0.70% DM-β-CD (w/w)

was prepared as follows, 200 mg SB-β-CD and 70 mg DM-β-CD were dissolved in 10.0 ml 25 mM phosphate buffer pH 8.0. The solution was then filtered through a filter (approx. 0.45 μm).

Electrolyte (for electrophoresis); 10/90 (v/v) %, ACN/buffer-electrolyte; 9.0 ml buffer-electrolyte added 1.0 ml ACN. The CE vials (e.g. 2.0 ml size HP 5181-3375) were filled with precisely 1.0 ml of electrolyte. The working life of the electrolyte is 6 months when stored in the freezer at around −20 °C.

Extraction solution (for extraction of samples and manufacture of standards): The extraction fluid consisted of acetonitrile (ACN) and 0.01 M NaOH in the ratio 20:80. Prepared by adding 200 ml ACN to 800 ml 0.01 M NaOH. The working life of this solution is 3 months.

One mole of ragaglitazar with arginine weighs 593.7 g, (ragaglitazar 419.5 g). All sample concentrations, stock solutions and dilutions are given in mass units, as the free acid.

2.2. Instrumentation

Capillary electrophoresis was carried out using an Agilent Technologies ^{3D}CE system (Agilent Technologies). Data acquisition and signal processing were performed using Agilent Technologies ^{3D}CE ChemStation (rev. A.06.03, Agilent Technologies).

The capillary was an 80.5 cm (72.0 cm efficient length) 50 μm i.d. "Extended Light Path Capillary" e.g. Agilent T. capillary HP part no. G1600-62232. UV detection was performed at 200 nm (16 nm Bw, Reference 350 nm and 80 nm Bw). The Auto sampler temperature was room temperature (approx. 21 °C). The running electrolyte was 10/90 (v/v) %, ACN/(2% SB-β-CD and 0.7% DM-β-CD in 25 mM phosphate buffer pH 8.0).

Preconditioning (for a new capillary): New capillaries were flushed with 1.0 M NaOH for 20 min followed by 20 min with 0.1 M NaOH. Preconditioning (for used capillaries) Used capillaries were conditioned for 10 min. with 0.1 M NaOH, then flushed with Milli-Q water for 10 min. Capillaries were rinsed after each sample run with 0.1 M NaOH and then water (flush for 1 min with each). Hydrodynamic injection at 40 mbar for 5.0 s (approx. 4 nl) was used. The voltage was +30 kV (50 μA approx.). The capillary temperature was 30 °C and runtime was 40 min.

2.3. Quality controls and standard solutions

The analysis concentration for samples and standards were 500 μg/ml for ragaglitazar as free acid and 208 μg/ml for arginine (corresponding to approx. 708 μg/ml ragaglitazar, arginine).

The SST solution (System Suitability Test for control of resolution) was prepared as follows: To a 20 ml measuring flask, approx. 3.5 mg ragaglitazar, arginine (eutomer) and 3.5 mg ragaglitazar, arginine (distomer) in 10 ml extraction solution (stability 14 days in refrigerator at 0–5 °C) were added. 20 μl of this solution was transferred to a CE-vial and 1 ml of extraction fluid was added. This System Suitability Test solution was injected three times before each run and once finally. This solution was used to evaluate the quality of the separation in the system by calculation of the resolution between ragaglitazar and its distomer.

Ragaglitazar, arginine standard (for quantitative determination and identification of ragaglitazar and arginine in the sample): This standard was prepared as a ragaglitazar, arginine standard as follows, in a 100 ml measuring flask, 70.8 mg ragaglitazar, arginine was dissolved in 90 ml extraction solution, and then, the flask was filled up to the mark with the extraction solution. This solution was prepared freshly each time and was used for quantitative determination of arginine in the sample.

This solution was also used to calculate the extraction efficiency of ragaglitazar from tablets and the identification of ragaglitazar and arginine in the CE system by comparing the relative migration time (RMt, calculated towards ragaglitazar) of ragaglitazar and arginine in samples.

Control solution (for control of recovery of standards): A control solution was prepared as described for standard. This solution was used for evaluating the recovery of the ragaglitazar and arginine in the standard during the analyses.

LOQ-solution of ragaglitazar (corresponding to approx. 0.10%): A ragaglitazar solution (standard or sample) was diluted 1000 times with extraction solution. This solution was used to control the sensitivity of the system at the start of each analysis.

Table 1
Sample preparation for ragaglitazar API and tablets

Sample	Flask (ml)	Sample amount	Extraction solution (ml)	Concentration of ragaglitazar as free acid (μg/ml)
Placebo tablets	10	4 tablets	4	0
0.5 mg tablets	10	4 tablets	4	500
1 mg tablets	10	4 tablets	8	500
2 mg tablets	20	4 tablets	16	500
API	20	10 mg	To the mark	500

2.4. Sample preparation

The analysis concentration for samples and standards was 500 μg/ml for ragaglitazar as the free acid and 208 μg/ml for arginine (corresponding to approx. 708 μg/ml ragaglitazar, arginine).

The sample solution was prepared according to Table 1. The tablets were transferred to the measuring flask, and the correct amount of extraction solution was added. The samples were stirred for approx. 60 min, centrifuged at 4000 rpm (with rotat radius of 15 cm) for 10 min and were then transferred directly to a CE-vial (ready for the analysis). The sample solutions were prepared fresh each time (Table 1).

2.5. Method conditions

This CE method is designed to provide analytical data for identification and assay of ragaglitazar and arginine, chiral purity and purity of ragaglitazar for both drug substance (API) and tablets. For this, the following points were considered for validation: Specificity and selectivity (matrix interference), linearity, range, accuracy, repeatability, intermediate precision, limit of detection, limit of quantification, robustness, and stability of solutions.

2.6. Sample degradation experiment

The stability indicating of the CE method was examined during the pre-validation.

This study was performed as a “worst case” study using an extreme stressed and degraded sample.

The sample was stressed as follow: A sample solution of a 2.0 mg tablet was kept at 100 °C for 120 h. Then, the sample was examined. Analysis of the sample showed that a possibility exists that degradation products can/will appear in the electropherogram up to 35 min after injection.

3. Results and discussion

3.1. Development of the CE method

The target of separation was ragaglitazar and its disomer. The effect of cyclodextrin type, cyclodextrin concentration, and addition of organic modifier to the electrolyte were investigated. A number of different cyclodextrins were tested to obtain the best separation. The studies showed that a combination of sulfobutylether-β-cyclodextrin and dimethyl-β-cyclodextrin gave a very good result. Then, the concentration of the two cyclodextrins in the electrolyte was optimized and found to be 2% for SB-β-CD and 0.7% for DM-β-CD. After choice and optimization of cyclodextrins, the long migration time of ragaglitazar (and its disomer) needed to be optimized. The effect of adding an organic modifier to the electrolyte was tested, and good results were achieved. Acetonitrile at a concentration of 10% (v/v) in the electrolyte was found to be the best choice as organic modifier.

Separation was not achieved for all known-related substances; however, a very good separation was achieved for ragaglitazar, distomer, arginine, and a large number of-related substances and degradation products.

During the stage of development and pre-validation, it was demonstrated that both rinse and pre-conditioning of the capillary improved the resolution, precision, and accuracy.

The use of 200 nm detection was a success, as it is also widely recommended and used in published CE methods [1–6,12–14].

3.2. Sample preparation development

The target of sample preparation was to reach a simple, practical and almost identical procedure for API and all tablets (0.5, 1.0 and 2.0 mg).

Different solutions with low, neutral and high pH and with or without organic solvents were tested. The target of these experiments was to extract ragaglitazar, the-related substances (including the distomer) and arginine with a good recovery. As a result an extraction solution containing 20% (v/v) acetonitrile and 80% (v/v) 0.01 M sodium hydroxide was chosen.

The sample preparation for both API and tablets is similar. The extraction procedure is simple, and a very good recovery is achieved for all compounds.

Changes in volume during sample preparation are exactly calculated during assay calculation for ragaglitazar and arginine in tablets.

3.3. Validation

The validation was performed with respect to selectivity, linearity, range, accuracy, repeatability and intermediate precision, limit of detection and quantification (LOD and LOQ), robustness, and stability of analytical solutions.

The validation was designed to cover all 12 analyses as 6 tests/analyses for the API and six test/analyses for the low-dose tablets (Table 2).

3.3.1. Selectivity

The method shows a significant degree of selectivity and ragaglitazar is well separated from its distomer and arginine (Fig. 2).

The separation of ragaglitazar, its distomer, arginine, and 22 known-related compounds was also studied. The result showed that some compounds were not separated, but 19 of these were separated and detected by the method (Fig. 3).

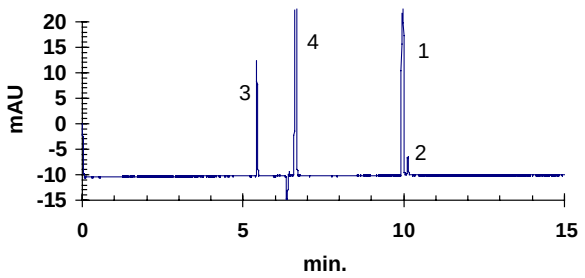

Fig. 2. CE separation of (1) ragaglitazar, (2) ragaglitazar distomer and (3) arginine (a counter ion of ragaglitazar) and (4) is the EOF.

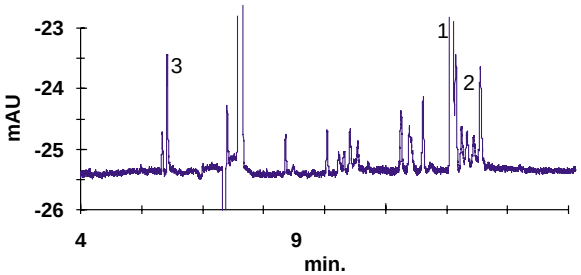

Fig. 3. CE separations of: ragaglitazar (1), ragaglitazar distomer (2), arginine (3) and 22 known-related compounds.

The runtime of the method is 40 min (for samples) and 15 min for standards. The 40 min runtime is normally too long for a CE method however, the runtime was chosen based on the results from the sample degradation experiment during pre-validation studies. The analysis of these samples showed that a possibility existed that degradation products can/will appear up to 35 min (migration time) post injection. This is shown in Fig. 4.

The sample degradation studies also showed that this CE method is a stability indicating method.

Peak purity and identity test was also performed using a diode array detector and by a spiking test procedure on both API and tablet test solutions. The purity and identification of the peaks were confirmed.

3.3.2. Linearity

A calibration curve for ragaglitazar and arginine was prepared using a placebo tablet solution with the addition of different volumes of a standard stock solution.

Linearity was tested at 2 different levels (1 and 2) for ragaglitazar and 1 level for arginine with 5 point at each level. Three injections were preformed at each point.

Level 1 was from 80 to 120% of the nominal concentration of active substance (500 μg ragaglitazar as free acid/ml and 208 μg/ml of arginine, corresponding to 708 μg/ml of ragaglitazar, arginine).

Level 2 was from 0.10 to 3.0% of the nominal concentration of ragaglitazar (1.0% is 5 μg ragaglitazar, as free acid/ml, corresponding to 7.08 μg/ml of ragaglitazar, arginine).

Table 2
Covering area of the CE method and its validation

Method no.	Test (and validation)	Selectivity	Linearity	Range	Accuracy	Repeatability	Intermediate Precision	LOD	LOQ	Robustness	Stability of Solutions
1, 2	Ragaglitazar identification	+	−	−	−	+	+	−	−	+	+
3, 4	Arginine identification	+	−	−	−	+	+	−	−	+	+
5, 6	Ragaglitazar assay	+	+	+	+	+	+	+	+	+	+
7, 8	Arginine assay	+	+	+	+	+	+	−	−	+	+
9, 10	Ragaglitazar chiral purity	+	+	+	+	+	+	+	+	+	+
11, 12	Ragaglitazar purity	+	+	+	+	+	+	+	+	+	+

Method nos. 1, 3, 5, 7, 9 and 11 are test methods for the API. Method nos. 2, 4, 6, 8, 10 and 12 are test methods for the low-dose tablets (0.5, 1.0 and 2.0 mg).

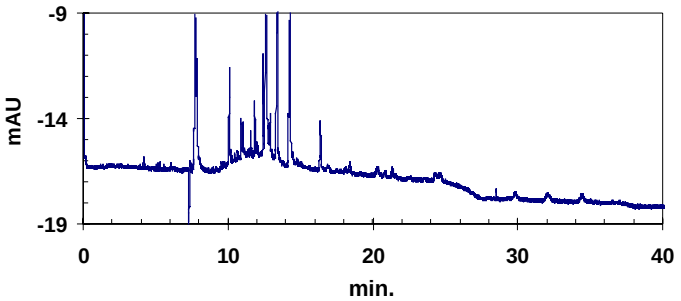

Fig. 4. Electropherogram of an extreme degraded sample (2 mg tablet sample solution, kept at 100 °C for 120 h).

The linearity of the method in each range was evaluated by linear regression analyses (area counts) using Excel Microsoft program (Table 3). All tested levels showed a good and acceptable linearity.

3.3.3. Range

The range of the CE method (injection volume was approximately 4 nl) for ragaglitazar were demonstrated at level 1 to be from 1603 to 2404 ng, level 2 to be from 0.2 to 60 ng, and for arginine at level 1 to be from 668 to 1000 ng.

3.3.4. Accuracy

The accuracy of the method was demonstrated as % recovery in levels 1 and 2 for ragaglitazar and level 1 for arginine with 5 points at each level. The recovery was calculated using a standard solution of ragaglitazar and arginine at each level.

Accuracy was tested using a placebo tablets and the reference standards. Placebo tablets were prepared as described in the sample preparation section and were then spiked with the reference standard solution.

The recovery of ragaglitazar was found to be between 101–106% at level 1 (80–120% of the nominal concentration) and 101–125% at level 2 (0.10–3.0% of the nominal concentration). The recovery of arginine was found to be between 97–101% at level 1 (80–120% of the nominal concentration).

Table 3
Linearity data obtain for ragaglitazar at levels 1 and 2, and arginine at level 1

Linear regression analysis ($Y = \beta X + \alpha$)	
Slope of the curve (β)	0.0038 (ragaglitazar level 1)
	0.0047 (ragaglitazar level 2)
	0.0009 (arginine 1)
Intercept (α)	0.1298 (ragaglitazar level 1)
	0.0010 (ragaglitazar level 2)
	−0.0023 (arginine level 1)
Squared correlation coefficient (r^2)	0.997 (ragaglitazar level 1)
	1.000 (ragaglitazar level 2)
	1.000 (arginine level 1)

3.3.5. Repeatability and intermediate precision

The precision of the method was determined in two steps, namely the repeatability and intermediate precision. To make the precision studies more realistic, highly stressed tablet batches were used.

The repeatability of the method was demonstrated by the mean value results, relative standard deviations of the determinations, and the confidence limits (95%). This was performed as 6 single determinations from one prepared solution (split in 6 portions/vials) at 100% level of the test concentration (500 μg ragaglitazar as free acid/ml and 208 μg arginine/ml).

For each study, 5 compounds/results were analyzed; ragaglitazar (as the free acid), distomer (the (+)-enantiomer), largest single impurity, total amount of impurities, and arginine (as % recovery of declared content). Results are noted in Table 4. The results showed a very good and acceptable repeatability.

The intermediate precision of the method was studied as follow.

On two different days, different operators prepared a test solution (one new sample preparation at each day) and performed six single determinations from the prepared solution (split in six portions/vials) at 100% level of the test concentration (500 μg ragaglitazar as free acid/ml and 208 μg arginine/ml). The analyses

Table 4
Repeatability of the CE method

Analysis no.	Result (%)				
	Ragaglitazar	Distomer	Largest single impurity	Total impurities	Arginine
1	94.95	2.00	1.22	5.05	97.4
2	94.90	2.01	1.22	5.10	97.8
3	94.96	1.98	1.24	5.04	99.6
4	95.04	1.96	1.34	4.96	100.4
5	94.95	2.01	1.35	5.05	102.6
6	94.97	1.98	1.39	5.03	101.3
Mean ($n = 6$)	94.96	1.99	1.29	5.04	100.0
%R.S.D. ($n = 6$)	0.05	1.01	5.84	0.90	2.0
95% Confidential level	±0.05	±0.02	±0.08	±0.05	±0.1

were preformed on two different CE instruments using two different batches of capillary.

For each study, five compounds/results were again analyzed; ragaglitazar (as free acid), distomer (the (+)-enantiomer), largest single impurity, total amount of impurities and arginine (as % recovery of declared content).

The intermediate precision of the method was demonstrated by the mean value results, relative standard deviations of the determinations performed in the repeatability and intermediate precision study and the confidence limits (95%) for both days. Results are noted in Table 5. The results showed a very good and acceptable intermediate precision.

3.3.6. Limit-of-quantitation (LOQ) and limit-of-detection (LOD)

The LOQ and LOD of the method were tested using the reference standard of ragaglitazar, arginine based on determination of the signal to noise ratio for the ragaglitazar peak. Signal to noise ratio for analysis of a 0.10% solution of ragaglitazar (of nominal concentration) was calculated using data obtained on different CE instruments. The results showed that 0.10% solu-

Table 5
Intermediate precision of the CE method

Analysis no.	Result (as %)				
	Ragaglitazar	Distomer	Largest single impurity	Total impurities	Arginine
1	94.95	2.00	1.22	5.05	97.4
2	94.90	2.01	1.22	5.10	97.8
3	94.96	1.98	1.24	5.04	99.6
4	95.04	1.96	1.34	4.96	100.4
5	94.95	2.01	1.35	5.05	102.6
6	94.97	1.98	1.39	5.03	101.3
7	96.26	2.04	1.38	3.74	106.5
8	96.01	2.04	1.38	3.99	105.2
9	96.08	2.04	1.38	3.92	106.0
10	95.98	2.03	1.37	4.02	105.2
11	96.20	2.07	1.37	3.80	107.8
12	95.99	2.10	1.45	4.01	106.5
Mean ($n = 12$)	95.52	2.02	1.34	4.48	103.0
%R.S.D. ($n = 12$)	0.63	1.98	5.22	13.17	3.50
95% Confidential level	±0.38	±0.03	±0.05	±0.38	±2.31

Data for analysis nos. 1–6 are from repeatability study and nos. 7–12 from the “second day” in intermediate precision study.

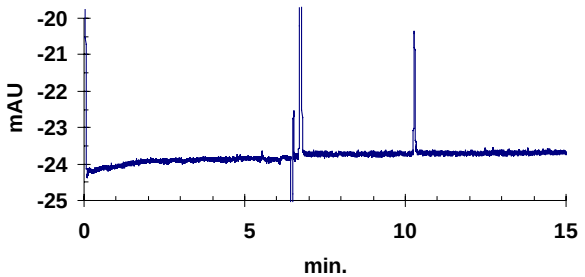

Fig. 5. Electropherogram of a LOQ ragaglitazar solution (0.10% of nominal concentration: 500 ng/ml, injection volume; 4 nl).

tion gave a very good signal to noise ratio, which was typically between 5:1 to 12:1 (depend on the age of the UV lamp) (Fig. 5).

Generally, the method shows a very low-baseline noise and high signal for ragaglitazar. It was accepted to have 0.05% as LOD and 0.10% as LOQ.

3.3.7. Robustness

The robustness of the method was examined during the development of the method and as a routine part of the validation. Parameters such as the concentration of the cyclodextrins, acetonitrile, different capillaries, different instruments and electrolyte ion strength capacity were examined. The resolution between the critical pair; ragaglitazar and the distomer was determined to evaluate the separation. For sample preparation the effect of stirring time was examined.

Generally, the method showed good robustness. Resolution between the critical pair, ragaglitazar and the distomer peak was maintained throughout the space around the method conditions, with cylodextrins and acetonitrile concentration being the only factors showing significant effects, which could easily be observed using a system suitability test solution when performing the analysis. The study also showed that ion strength capacity of electrolyte is good, but a time limited period of 90 min (two 40 min runs) will show the best result.

Changes in up to 25% in stirring time for sample preparation showed no effect on the results.

3.3.8. Stability of analytical solutions

The stability of sample solution, standard solutions, extraction solution, and electrolyte was examined.

The sample solution and arginine standard solution were found to be stable when stored in a tightly closed container in a refrigerator (0–5 °C) for 4 days. All standard solutions containing ragaglitazar were found to be stable when stored in a tightly closed container at refrigerator for 14 days. The extraction solution was found to be stable when stored in a tightly closed container at refrigerator for 3 months. The electrolyte was found to be stable when stored in a tightly closed container at refrigerator (0–5 °C) for 6 months.

4. Conclusion

A selective, sensitive and robust capillary electrophoresis (CE) method has been developed and validated for multi determination analysis of ragaglitazar (NNC 61-0029 or DRF 2725) and its counter ion arginine in API and low-dose tablets (0.5, 1.0 and 2.0 mg). The method covers analyses for the API and tablets: assay and identification of ragaglitazar and arginine, chiral purity of ragaglitazar and purity of ragaglitazar (12 tests/analyses).

Good and acceptable method performance has been demonstrated for all validation points. Ragaglitazar, arginine, potential-related substances, degradation products, and chiral-related substance can be reliably identified, verified and quantified at 0.10% level of the drug substance with good precision. Arginine (the counter ion of ragaglitazar) was well separated and detected in the same method and could be identified and quantified in the API and low-dose tablets.

In general, the results demonstrate that CE can be applied as a multi method and a complementary and/or alternative technique to e.g. HPLC for pharmaceutical analyses at R&D.

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