Estimation of *(R)*-Isomer and Other Impurities of Zolmitriptan API by Capillary Zone Electrophoresis

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Overview

Who Should Read This: Senior Scientists, Lab Directors

Focus: Measurement of the *(R)*-isomer and impurities of zolmitriptan by capillary zone electrophoresis (CZE).

Goals: Successfully apply the United States Pharmacopoeia (USP) capillary electrophoresis method for determination of zolmitriptan enantiomers using the SCIEX P/ACE™ MDQ Plus Capillary Electrophoresis System. Meet established USP standards.

Problem: Zolmitriptan, (4S)-4-[3-[2-dimethyl aminoethyl]-1*H*-5-indolyl-methyl]-1,3-oxazolan-2-one, is a serotonin receptor agonist that is highly effective in the acute treatment of migraines.[1] Zolmitriptan is synthesized as the *(S)*-isomer because *(S)*-zolmitriptan is pharmacologically more potent than *(R)*-zolmitriptan and because *(R)*-zolmitriptan is toxic. Due to this toxicity, the allowed limit of the *(R)*-isomer has been established by the USP as 0.15% w/w.[2] As such, an accurate and robust method is needed to evaluate the enantiomeric purity of zolmitriptan.

Results: The system met the suitability standards established by the United States Pharmacopoeia. Obtained relative migration times (RMTs) were comparable to USP RMTs and consistent over multiple runs.

Key Challenges:

- The developed analytical method must be able to resolve the enantiomers of zolmitriptan as well as related impurities
- The developed analytical method must be accurate and reproducible

Figure 1: The SCIEX P/ACE™ MDQ Plus Capillary Electrophoresis (CE) System

Key Features:

- Capillary zone electrophoresis is highly effective at separating enantiomers
- In many cases, capillary zone electrophoresis provides greater resolution than liquid chromatography
- The P/ACE™ MDQ Plus Capillary Electrophoresis (CE) System features, as standard, temperature control of both the sample and capillary. This greatly enhances the analytical reproducibility that is crucial for quality control applications.

Experimental Design

Materials

Table 1. Chemical supplies

Reagent/material	Catalog Number	Vendor
Zolmitriptan	1727009	United States Pharmacopoeia (USP)
Zolmitriptan *(R)*-isomer	1727018	United States Pharmacopoeia (USP)
Zolmitriptan Related Compound F	1727075	United States Pharmacopoeia (USP)
Zolmitriptan Related Compound GS	1727086	United States Pharmacopoeia (USP)
Sodium borate	B3545	Sigma
Tryptamine HCl-A030	F1101-RMBB	www.tcichemicals.com
Hydroxypropyl β-cyclodextrin	XFR3G-MS	www.tcichemicals.com
Orthophosphoric acid, 88%	ECOA600144	Merck
Hydrochloric acid	HK3H530649	Merck
Water, Purified – Type 1	Milli-Q	Millipore

Table 2: Vials, parts, and other supplies

Material	Catalog Number	Vendor
Universal vials (pkg of 100)	A62251	SCIEX
Universal vial caps (pkg of 100)	A62250	SCIEX
nanoVial (qty 100)	5043467	SCIEX
Capillary – 75 µm ID, 111 cm total length (qty 3)	360800	SCIEX
Cartridge assembly kit, blank	144738	SCIEX
15 & 50 mL conical bottom tubes	NA	Appropriate third party
Assorted pipettes and corresponding tips	NA	Appropriate third party
Microcentrifuge vials	NA	Appropriate third party
3 mL syringe with needle	NA	Appropriate third party
0.5 mL centrifuge vials	NA	Appropriate third party
0.45 µm syringe filters	4497	Pall Corporation

Reagent and Sample Preparation[3, 4]

Stock Solutions

Internal standard stock solution

Tryptamine was used as the internal standard (IS). The tryptamine internal standard stock solution was prepared by dissolving 2.0 mg of tryptamine in 0.02 M HCl made to a total volume of 10 mL.

Zolmitriptan stock solutions

The zolmitriptan stock solution was prepared by dissolving 2 mg of zolmitriptan in 0.02 M HCl made to a total volume of 1 mL. Similarly, zolmitriptan F, G, and *(R)*-isomer stock solutions were each prepared (separately) by dissolving 1 mg of the compound in 1 mL of 0.02 M HCl.

Working Solutions

System suitability solution (0.01 mg/mL tryptamine, zolmitriptan F, zolmitriptan G, and zolmitriptan *(R)*-isomer + 1 mg/mL zolmitriptan)

The system suitability solution was prepared by adding 10 µL of each stock solution (zolmitriptan F, G, and *(R)*-isomer), 50 µL of tryptamine (IS) stock solution, and 500 µL of zolmitriptan RS stock solution to a 2 mL microcentrifuge tube. The volume was made up to 1000 µL using 0.02 M HCl solution. The final mixture contained 1.0 mg/mL of zolmitriptan, 0.01 mg/mL of zolmitriptan F, G and *(R)*-isomer impurities and 0.01 mg/mL of tryptamine (IS).

Standard solution (0.01 mg/mL of tryptamine, 0.001 mg/mL zolmitriptan)

The standard solution was prepared by first diluting 50 µL of zolmitriptan stock solution (2 mg/mL) with 950 µL of 0.02 M HCl to produce a 0.1 mg/mL zolmitriptan solution. 10 µL of the 0.1 mg/mL zolmitriptan solution and 50 µL of the tryptamine (IS) stock (0.2 mg/mL) were then aliquoted into a microcentrifuge tube, to which was added 940 µL of 0.02 M HCl. The solution was gently vortexed for uniform mixing.

Sample solution (0.01 mg/mL of tryptamine, 1.0 mg/mL zolmitriptan sample)

1 mg of sample was weighed into a 1.5 mL Eppendorf tube. 950 µL of 0.02 M HCl and 50 µL of tryptamine (IS) stock solution were added and briefly vortexed to dissolve.

CZE running buffer

286.5 mg sodium borate decahydrate and 7 mL of water were combined in a 15 mL polypropylene tube and briefly vortexed to dissolve the mixture. The pH was adjusted to 2.2 with 1 N orthophosphoric acid. 750 mg of β-cyclodextrin was added to the mixture and vortexed to dissolve. Water was added to bring the total solution volume to 15 mL. The solution was filtered through a 0.45 µm syringe filter.

System Set Up and Configuration

All experiments were performed on a P/ACE™ MDQ Plus Capillary Electrophoresis (CE) System (SCIEX, Framingham, USA), equipped with a UV detector and 200 nm filter. The capillary was a 75 µm ID bare fused silica capillary with 50 cm effective length. The cartridge detection window aperture was 100 x 200 µm. The instrument was controlled by 32 Karat™ software version 10.1.33.

Initial Conditions

The initial conditions were the same for all methods:

Capillary/Sample Storage/Peak Detection Initial Conditions (see also Figure 2)

- Voltage maximum: 30.0 kV
- Current maximum: 300 µA
- Cartridge temperature: 20.0° C
- Sample storage: 15.0° C.
- Peak detection threshold: 2
- Peak width: 9
- Analog output scaling: 1

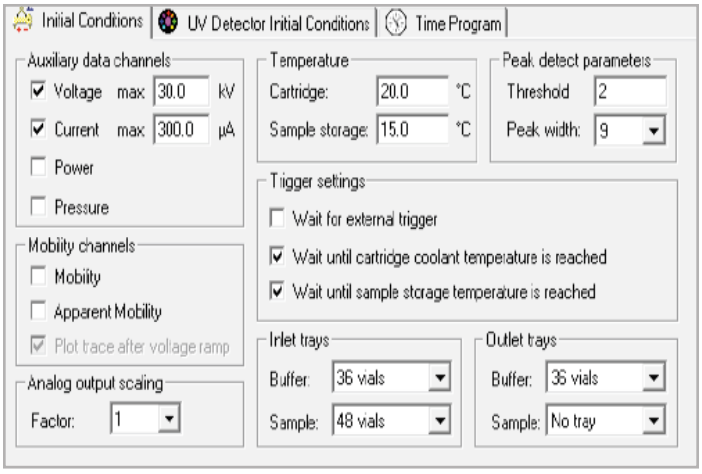

Figure 2: Initial conditions for capillary, sample storage, and peak detection

UV Detector Initial Conditions (see also Figure 3)

- Acquisition: Enabled
- Wavelength of 200 nm
- Data rate: 4 Hz
- Filter: Normal
- Peak width (points): 16–25
- Absorbance signal: Direct

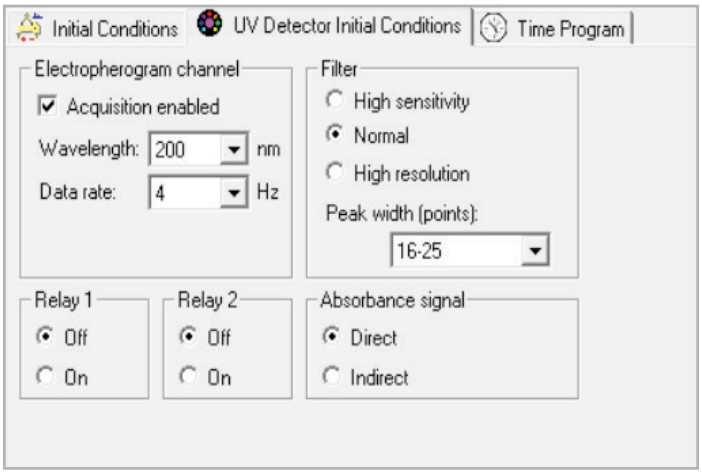

Figure 3: Initial conditions for the UV detector

Current

The current generated throughout the separation was between 70 µA and 90 µA.

Separation Methods

Three methods were created in 32 Karat™ software. They were: capillary equilibration, zolmitriptan separation, and shutdown (programs depicted in Figures 4, 5, and 6, respectively).

Capillary equilibration was performed as follows:

1. 30 min rinse at 20 psi of 0.1 N NaOH
2. 60 min rinse at 20 psi of 0.1 N HCl
3. 60 min rinse at 20 psi of 0.1 N orthophosphoric acid
4. 60 min rinse at 20 psi of running buffer
5. Voltage equilibrium of 15 kV for 6 hours

Zolmitriptan separation was achieved as follows:

1. 3 min rinse at 20 psi of water
2. 5 min rinse at 20 psi of 0.1 N orthophosphoric acid
3. 5 min rinse at 20 psi with 0.1 N HCl
4. 7 min rinse and fill at 20 psi using running buffer
5. Hydrodynamic sample introduction at 1.0 psi for 5 secs
6. Buffer plug at 0.5 psi for 5 sec
7. Separation at 15.0 kV for 40 min (250 V/cm)

Initial Conditions | UV Detector Initial Conditions | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	20.0 psi	30.00 min	BI:B6	BO:B6	forward	0.1N NaOH rinse
2		Rinse - Pressure	20.0 psi	60.00 min	BI:E6	BO:E6	forward	0.1NHcl Rinse
3		Rinse - Pressure	20.0 psi	60.00 min	BI:F6	BO:F6	forward	0.1N ortho phosphoric acid rinse
4		Rinse - Pressure	20.0 psi	60.00 min	BI:D6	BO:D6	forward	BGE Rinse and fill
5	0.00	Separate - Voltage	15.0 KV	360.00 min	BI:C6	BO:C6	5.00 Min ramp, normal polarity	Conditioning by voltage
6	5.00	Autozero						
7	360.00	End						
8								

Figure 4: Capillary equilibrium time program

Zolmitriptan separation was achieved as follows:

1. 3 min rinse at 20 psi of water
2. 5 min rinse at 20 psi of 0.1 N orthophosphoric acid
3. 5 min rinse at 20 psi with 0.1 N HCl
4. 7 min rinse and fill at 20 psi using running buffer
5. Hydrodynamic sample introduction at 1.0 psi for 5 secs
6. Buffer plug at 0.5 psi for 5 sec
7. Separation at 15.0 kV for 40 min (250 V/cm)

Initial Conditions | UV Detector Initial Conditions | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	20.0 psi	3.00 min	BI:A1	BO:B1	forward, In / Out vial inc 7	Water Rinse
2		Rinse - Pressure	20.0 psi	5.00 min	BI:F1	BO:F1	forward, In / Out vial inc 7	0.1N O Phosphoric Acid
3		Rinse - Pressure	20.0 psi	5.00 min	BI:E1	BO:E1	forward, In / Out vial inc 7	0.1N Hcl Rinse
4		Rinse - Pressure	20.0 psi	7.00 min	BI:D1	BO:D1	forward, In / Out vial inc 7	BGE Rinse and Fill
5		Inject - Pressure	1.0 psi	5.0 sec	SI:A1	BO:C1	Override, forward	Sample Inject
6		Inject - Pressure	0.5 psi	5.0 sec	BI:B1	BO:C1	No override, forward, In / Out vial inc 7	Water Plug
7		Wait		0.00 min	BI:A4	BO:A4	In / Out vial inc 7	Capillary ends Wash
8	0.00	Separate - Voltage	15.0 KV	40.00 min	BI:C1	BO:C1	0.17 Min ramp, normal polarity, In / Out vial inc 7	Separation
9	5.00	Autozero						
10	40.10	End						
11								

Figure 5: Zolmitriptan separation time program

Initial Conditions | UV Detector Initial Conditions | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1	0.00	Separate - Pressure	30.0 psi	5.00 min	BI:B1	BO:B1	forward	Water Rinse
2	5.01	Wait		0.00 min	BI:B1	BO:A1		
3	5.05	Lamp - Off						
4	5.06	End						

Figure 6: Shutdown time program

Peak Integration

To take full advantage of the software's ability to analyze data as it is acquired, a few parameters need to be set in the 32 Karat™ software. The integration parameters optimized for the analysis of Zolmitriptan are shown in Figure 7.

#		Event	Start Time	Stop Time	Value
1	☑	Width	0.000	0.000	0.5
2	☑	Threshold	0.000	0.000	2500
3	☑	Integration Off	0.000	15	0
4	☑				

Figure 7: Peak integration parameters for analysis of zolmitriptan

Results and Discussion

System Suitability

Per United States Pharmacopoeia, the system suitability solution must be run with a minimum of 3 replicates and standard solution with a minimum of 6 replicates in each sequence or batch.

Resolution should be not less than 1.5 between zolmitriptan and zolmitriptan *(R)*-isomer peaks in all of the system suitability solution injections.

Relative standard deviation should be not more than 5% for the peak response ratio of zolmitriptan and tryptamine peaks in the replicate standard solution injections.

Figure 8 shows an electropherogram generated by analysis of the system suitability solution – 0.01 mg/mL, zolmitriptan *(R)*-isomer, zolmitriptan related compound F, zolmitriptan related compound G, tryptamine; 1 mg/mL zolmitriptan.

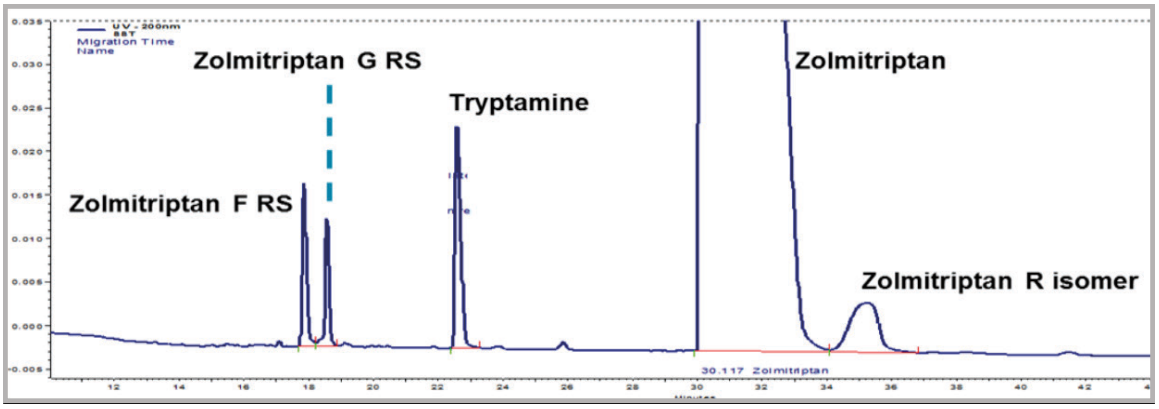

Figure 8: Electropherogram of system suitability solution

The Peak ID table was set as shown in Figure 9 so the software could annotate the peaks.

Named Peaks | Groups

#		Name	ID	Mig. Time	MT Window	Ref. ID #	ISTD. ID #
1	☑	Zolmitriptan related Comp F	1	18.05	0.9025	0	0
2	☑	Zolmitriptan related Comp G	2	18.7542	0.937708	0	0
3	☑	Tryptamine	3	22.8083	1.14042	0	0
4	☑	Zolmitriptan	4	30.5	1.525	0	0
5	☑	Zolmitriptan R isomer	5	35.5542	1.77771	0	0
6	☑						

Figure 9: Peak ID table.

Table 3 lists the resolution between zolmitriptan, zolmitriptan *(R)*-isomer, zolmitriptan related compounds, and the internal standard. The system passed the USP system suitability requirements.

Name	Migration Time (MT)	Relative MT	Resolution (USP)	Area	Corrected Area
Zolmitriptan Related Compound F	17.183	0.611	0.000	140359	5473
Zolmitriptan Related Compound G	17.738	0.631	2.824	86931	3284
Tryptamine	21.262	0.756	15.095	281104	8858
Zolmitriptan	28.108	1.000	4.306	22747153	542209
Zolmitriptan (R)-Isomer	32.779	1.166	**2.12**	275765	5637

Table 3: Result table of system suitability injections. Highlighted (bold) is the resolution of zolmitriptan *(R)*-isomer with respect to zolmitriptan. USP minimum is 1.5.

Figure 10 shows an overlay of 6 replicates of standard solution injections. Table 4 presents the numerical results. The system passed the USP requirements.

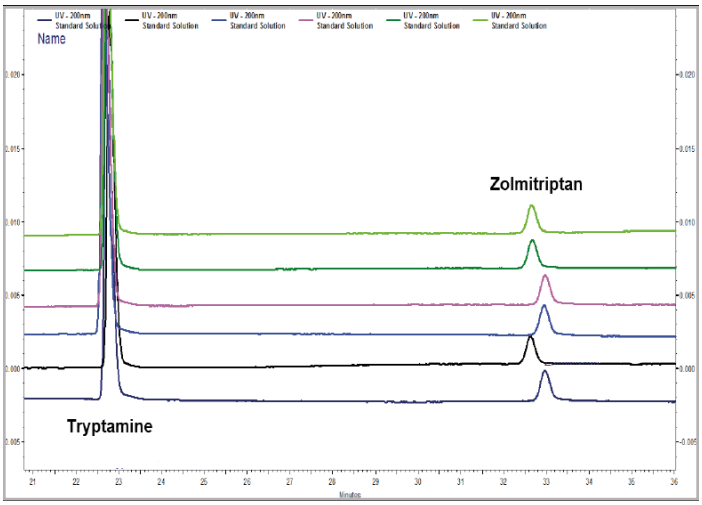

Figure 10: Overlay of 6 replicate injections of standard solution.

Sample Number	Name	Area	Corrected Area	Peak Response Ratio Area	Peak Response Ratio Corrected Area	Area SD	Area % RSD	Corrected Area SD	Corrected Area % RSD
1	Tryptamine	313601	9229	10.243	14.838	0.2	**2.4**	0.3	**1.9**
	Zolmitriptan	30616	622						
2	Tryptamine	306957	9009	9.735	14.143				
	Zolmitriptan	31530	637						
3	Tryptamine	305398	8968	9.831	14.280				
	Zolmitriptan	31065	628						
4	Tryptamine	297059	8757	10.192	14.644				
	Zolmitriptan	29145	598						
5	Tryptamine	294063	8665	10.214	14.686				
	Zolmitriptan	28789	590						
6	Tryptamine	291943	8625	10.287	14.744				
	Zolmitriptan	28380	585						

Table 4: Results from 6 replicate injections of standard solution. Area and corrected area %RSDs are highlighted (bold). USP maximum is 5%.

Sample Analysis

Data from the standard solution and sample solution were processed using the following formula to calculate the corrected peak responses:

Corrected Peak Response = (r/m)

Where:

r = peak response

m = migration time of the peak (min)

The percentage of each impurity was calculated by:

% Impurity = (R_u/R_S) X (C_s/C_u) X (1/F) X 100

Where:

R_u = corrected peak response ratio of the impurity to the internal standard from the *sample solution*

R_S = corrected peak response ratio of zolmitriptan to the internal standard from the *standard solution*

C_S = concentration of USP zolmitriptan in the *standard solution* (mg/mL)

C_U = concentration of zolmitriptan in the *sample solution* (mg/mL)

F = relative response factor for the corresponding impurity peak

Name	Relative Migration Time	Relative Response Factor	Acceptance Criteria NMT (%)
Zolmitriptan Related Compound F	0.68*	0.39	1.2
Zolmitriptan Related Compound G	0.71*	0.63	0.1
Tryptamine	0.78*	1.0	-
Zolmitriptan	1.0	-	-
Zolmitriptan (R)-Isomer	1.07*	1.0	0.2
Any individual unspecified impurity	-	1.0	0.1

* RMTs will vary depending on the pH, concentration of separation buffer, conditioning and length of capillary.
Disregard peak due to Zolmitriptan related compound E.
Disregard peaks less than 0.10% of the area of the principal peak from the sample solution.

Table 5: Acceptance criteria as per USP 39–NF 34 (8184)

Conclusion

Separation and measurement of zolmitriptan enantiomers are necessary for quality control and for related pharmaceutical and biological study of this drug. Capillary electrophoresis technology can be readily applied to determine enantiomeric purity of zolmitriptan, even in the presence of its potential process-related impurities. Using the United States Pharmacopoeia CZE method, the P/ACE™ MDQ Plus System provided the resolution and reproducibility needed to separate the zolmitriptan enantiomers and meet USP requirements.

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