Estimation of Almotriptan-Related Impurities by Capillary Zone Electrophoresis

SCIEX PA 800 Plus Pharmaceutical Analysis System

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Overview

Almotriptan malate is a selective serotonin 5-HT1B/1D agonist belonging to the class of second-generation triptans. It is used for acute treatment of the headache phase of migraine attacks[1]. Impurities commonly associated with almotriptan malate include: almotriptan N-dimer and almotriptan-related impurities B, C, and D [2]. For quality control, pharmaceutical research, and biological studies, it is essential that researchers have an accurate and robust method for detecting and measuring these impurities to ensure the almotriptan meets established standards. Capillary electrophoresis features outstanding resolving power and demonstrated robustness, repeatability, and reproducibility. It can readily be applied to detect almotriptan impurities.

This note demonstrates measurement of almotriptan and almotriptan impurities by capillary zone electrophoresis (CZE). It shows successful execution of the United States Pharmacopoeia (USP) capillary electrophoresis method for estimation of almotriptan impurities on a SCIEX PA 800 Plus Pharmaceutical Analysis System. The system met the suitability standards established by the USP. Obtained relative migration times (RMTs) were comparable to USP RMTs and consistent over multiple runs.

Key Features

- Capillary zone electrophoresis offers excellent compound resolution and is highly effective at separating impurities
- In many cases, capillary electrophoresis can provide greater resolution than liquid chromatography
- With a well-designed method, capillary electrophoresis also has very good repeatability and reproducibility
- The PA 800 Plus Pharmaceutical Analysis System features, as standard, temperature control of both the sample and capillary, greatly enhancing analytical reproducibility

Figure 1. The SCIEX PA 800 Plus Pharmaceutical Analysis System

Table 1. Results from 6 replicate injections of standard solution. % RSD is highlighted (bold). USP maximum is 5%.

4-Hydroxy-4-phenylpiperidine (IS)			Almotriptan			IC/Ref STD
#	RT	Area	#	RT	Area	
1	25.521	8095	1	32.742	11916	0.68
2	25.546	9852	2	32.742	14087	0.7
3	25.508	9928	3	32.675	15010	0.66
4	25.433	9942	4	32.567	14075	0.71
5	25.229	13539	5	32.179	19782	0.68
6	25.225	11712	6	32.217	16440	0.71
		% RSD				**2.8**

Experimental

Materials

Table 2. Chemical supplies

Reagent/Material	Catalog Number	Vendor
Almotriptan malate RS	*1013512*	*USP*
Almotriptan-related compound B	*1013545*	*USP*
Almotriptan-related compound C	*1013556*	*USP*
Almotriptan-related compound D	*1013567*	*USP*
4-Hydroxy-4-phenylpiperidine	*H 52201*	*Merck*
Phosphoric acid	*W 290017*	*Merck*
Triethanolamine	*90279*	*Merck*
Sodium hydroxide	*S 8045*	*Merck*
Methanol	*34860*	*Merck*
HPLC Grade water	*34877*	*Merck*

Table 3. 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	360800	SCIEX
Cartridge assembly	144738	SCIEX
15 & 50 mL conical-bottom tubes	NA	NA
Assorted pipettes and tips	NA	NA
Microcentrifuge vials	NA	NA
3 mL syringe with needle	NA	NA
0.5 mL centrifuge vials	NA	NA
0.45 µm syringe filters	4497	Pall Corp

Reagent and Sample Preparation

Internal standard stock solution: The 4-hydroxy-4-phenylpiperidine internal standard stock solution was prepared by dissolving 0.1 mg of 4-hydroxy-4-phenylpiperidine in 1:1 methanol:water (diluent) made to a total volume of 10 mL.

Standard stock solutions: Almotriptan stock solution was prepared by dissolving 0.5 mg of USP almotriptan malate RS in 1:1 methanol:water, made to a total volume of 1 mL.

Standard solution: Standard stock solution was prepared by diluting almotriptan standard stock solution in internal standard solution to a final concentration 0.005 mg/mL. This was filtered by passing through a suitable filter of 0.45 µm pore size.

System suitability solution: System suitability solution was prepared by diluting almotriptan-related compounds B, D, and RS, and almotriptan standard stock solution in internal standard solution to a final concentration 0.005 mg/mL. This was filtered by passing through a suitable filter of 0.45 µm pore size. Almotriptan N-dimer was not available for this experiment.

Sample solution: Sample solution was prepared by dissolving 2.5 mg of almotriptan malate in 1 mL of internal standard solution. The solution was sonicated to promote dissolution and passed through a suitable filter of 0.45 µm pore size.

CZE running buffer: Phosphoric acid was dissolved in water to a concentration of 23.5 g/L. The pH of the solution was adjusted to 3.0 using triethanolamine (TEA). This was filtered by passing through a suitable filter of 0.45 µm pore size.

System Set Up and Configuration

All experiments were performed on a PA 800 Plus Pharmaceutical Analysis System (SCIEX, Framingham, MA, USA), equipped with a UV detector and 214 nm filter. The capillary was a 75 µm ID uncoated fused silica capillary with 60 cm total length. The cartridge detection window aperture was 100 x 200 µm. The instrument was controlled by 32 Karat™ software.

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

Initial conditions for the capillary, sample storage, and peak detection are listed below and in Figure 2.

Voltage maximum: 30.0 kV;
Current maximum: 300 µA;
Cartridge temperature: 25.0° C;
Sample storage: 15.0° C;
Peak detection threshold: 2
Peak width: 9
Analog output scaling: 1

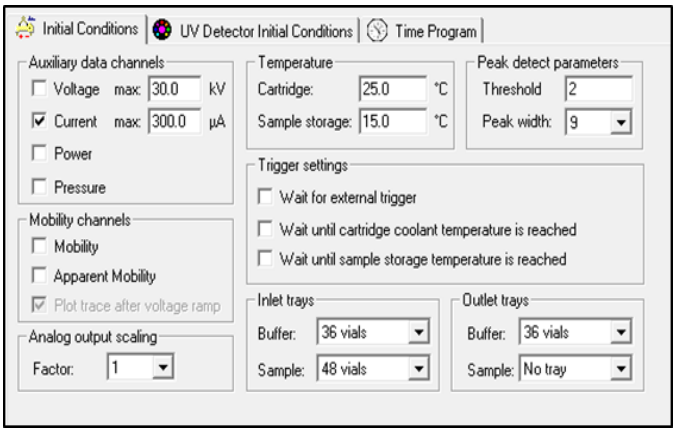

Figure 2. Initial conditions for capillary, sample storage, and peak detection.

UV Detector Initial Conditions (Figure 3)

Initial conditions for the UV detector are listed below and in Figure 3.

Acquisition: Enabled
Wavelength of 214 nm
Data rate: 4 Hz
Filter: Normal
Peak width (points): 16–25
Absorbance signal: Direct

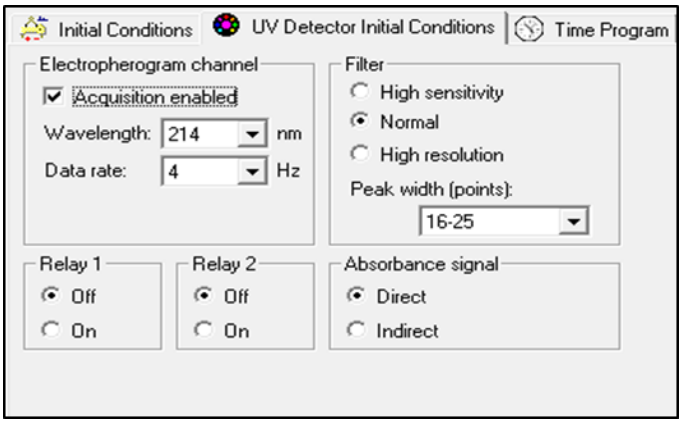

Figure 3. Initial conditions for the UV detector.

Separation Methods

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

Capillary equilibration (Figure 4)

1. Rinse with water at 20 psi for 5 min
2. Rinse with 0.1 N NaOH at 20 psi for 10 min
3. Rinse with run buffer (background electrolyte, BGE) at 20 psi for 10 min rinse
4. Voltage equilibrium at 15 kV for 30 min

Almotriptan separation (Figure 5)

1. Rinse with water at 20 psi for 3 min
2. Rinse with 0.1 N NaOH at 20 psi for 5 min
3. Rinse with run buffer (background electrolyte, BGE) at 20 psi for 7 min
4. Hydrodynamic sample introduction at 0.5 psi for 8 secs
5. Buffer plug at 0.5 psi for 3 secs
6. Separation at 15.0 kV for 45 min (250 V/cm)

Peak Integration (Figure 7)

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 almotriptan are shown in Figure 7.

Data Analysis

Data from the standard solution and sample solution were processed using the following formula to calculate the corrected peak responses2. Acceptance criteria are listed in Table 4.

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 = $(R_u/R_S) \times (C_s/C_u) \times 100$
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 almotriptan to the internal standard from the standard solution
C_S = concentration of USP almotriptan malate RS in the standard solution (mg/mL)
C_U = concentration of USP almotriptan malate RS in the sample

Initial Conditions | UV Detector Initial Conditions | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	
1		Rinse - Pressure	20.0 psi	5.00 min	BI:A1	BO:B1	forward	Water Rinse
2		Rinse - Pressure	20.0 psi	10.00 min	BI:D1	BO:D1	forward	0.1N NaOH Rinse
3		Rinse - Pressure	20.0 psi	10.00 min	BI:B1	BO:B1	forward	BGE Rinse
4	0.00	Separate - Voltage	15.0 KV	30.00 min	BI:C1	BO:C1	5.00 Min ramp, normal polarity	Voltage Equilibrium
5	5.00	Autozero						
6	30.00	End						
7								

Figure 4. Capillary equilibrium method

Initial Conditions | UV Detector Initial Conditions | Time Program

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	
1		Rinse - Pressure	20.0 psi	3.00 min	BI:A1	BO:B1	forward	Water Rinse
2		Rinse - Pressure	20.0 psi	5.00 min	BI:D1	BO:D1	forward	0.1N NaOH Rinse
3		Rinse - Pressure	20.0 psi	7.00 min	BI:B1	BO:B1	forward	BGE Rinse
4		Inject - Pressure	0.5 psi	8.0 sec	SI:A1	BO:C1	Override, forward	Sample Injection
5		Inject - Pressure	0.5 psi	3.0 sec	BI:B2	BO:C1	No override, forward	Buffer Injection/Plug
6	0.00	Separate - Voltage	15.0 KV	45.00 min	BI:C1	BO:C1	0.17 Min ramp, normal polarity	Voltage Equilibrium
7	1.00	Autozero						
8	45.00	End						
9								

Figure 5. Almotriptan separation method

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. Shut down method

#		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 almotriptan

Table 4. Acceptance criteria, as per USP IRA-1-May-2017

Name	Relative MT	Acceptance Criteria NMT (%)
Almotriptan N-dimer	0.71	0.3
Internal standard	0.78	-
Almotriptan related compound B	0.92	-
Almotriptan	1.00	-
Almotriptan related compound C	1.02	-
Almotriptan related compound D	1.22	0.1
Any individual unspecified impurities	-	0.1

Results and Discussion

As per United States Pharmacopoeia, the resolution should be not less than 2.0 between almotriptan-related compound B and almotriptan peaks and should not be less than 2.0 between almotriptan-related compound D and almotriptan peaks in all the system suitability solution injections. Relative standard deviation should not be more than 5.0% for the peak response ratio of almotriptan and 4-hydroxy-4-phenylpiperidine (internal standard) peaks in the replicate standard solution injections. Figure 8 shows an electropherogram generated by analysis of the 0.005 mg/mL system suitability solution containing almotriptan related-compound B, almotriptan-related compound D, 4-hydroxy-4-phenylpiperidine (internal standard), and almotriptan RS.

Table 1 lists the resolution between almotriptan standard, almotriptan-related compound B, almotriptan-related compound D, and the internal standard. The system passed the USP system suitability requirements. Figure 9 shows an overlay of 6 replicates of standard solution injections. Table 1 presents the numerical results. The system passed the USP requirements.

Figure 8: Electropherogram of system suitability solution.

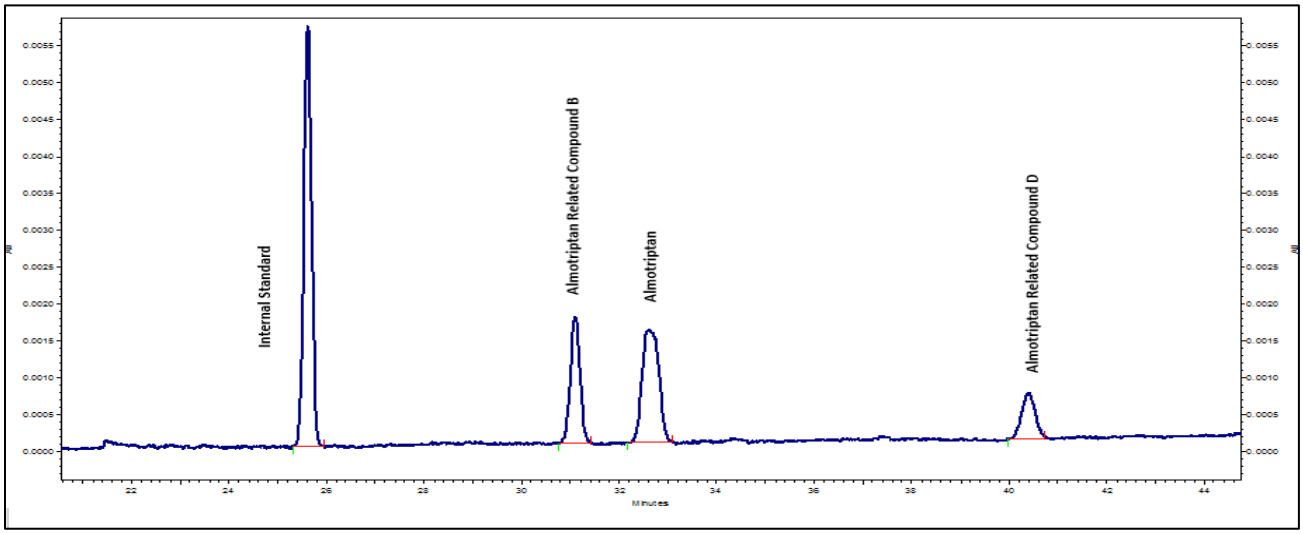

Table 5. Results of system suitability injections

Injection Number	Sample Name	Migration Time (MT)	Relative MT	Corrected Area	Resolution (USP)	Area ratio of IS/Standard
1	Internal standard	25.354	0.78	15198	0.00	0.33
	Almotriptan- related compound B	30.942	0.95	24474	16.6	
	Almotriptan standard	32.575	1.00	46054	3.4	
	Almotriptan-related compound D	40.167	1.23	27268	13.7	
2	Internal standard	25.321	0.78	13708	0.00	0.325
	Almotriptan-related compound B	30.875	0.95	21866	16.6	
	Almotriptan standard	32.508	1.00	42214	3.4	
	Almotriptan-related compound D	40.296	1.24	25402	13.9	
3	Internal standard	25.467	0.78	8166	0.00	0.326
	Almotriptan-related compound B	31.133	0.95	12874	16.3	
	Almotriptan standard	32.817	1	25033	3.4	
	Almotriptan- related compound D	40.808	1.24	14723	13.7	
		% RSD				**0.83**

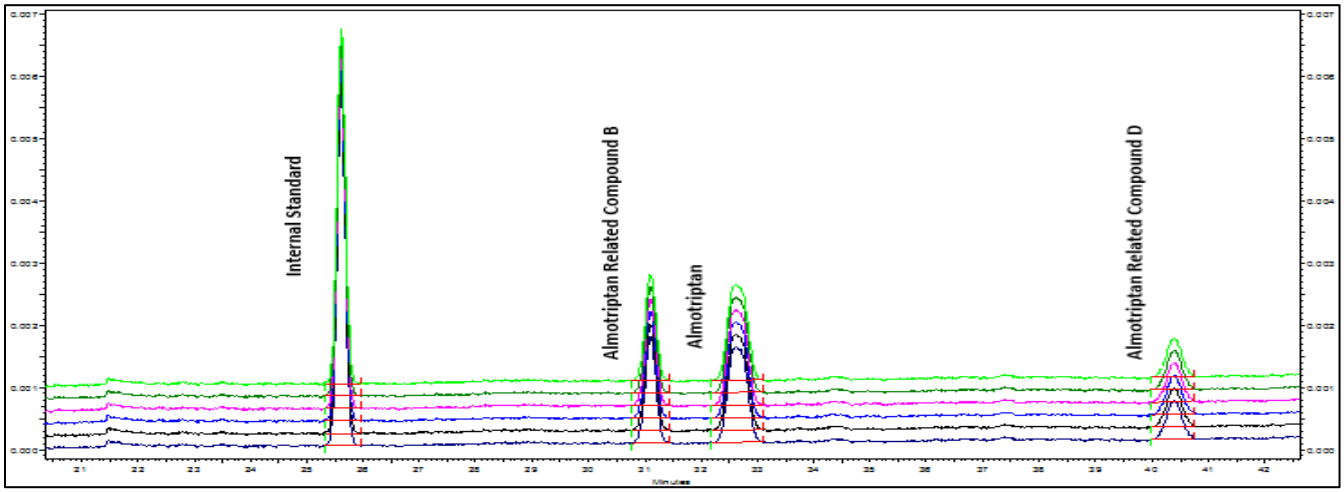

Figure 9. Overlay of 6 replicate injections of system suitability solution.

Conclusion

Separation and measurement of almotriptan impurities is necessary for quality control and for pharmaceutical and biological study of this drug. Capillary electrophoresis technology can readily be applied to determine almotriptan impurities. This study, demonstrated the measurement of almotriptan-related impurities B and D. Using the United States Pharmacopoeia capillary electrophoresis method, the PA 800 Plus Pharmaceutical Analysis System provided the resolution and reproducibility needed to separate the impurities and met the USP suitability standards. Obtained relative migration times (RMTs) were comparable to USP RMTs and consistent over multiple runs.

References

1. Orlandini S, *Journal of Chromatography*. **2014**. 1339 (200-209)
2. Almotriptan / *USP Official Monograph, Interim Revision Announcement*. Official May 1, 2017

Document number: RUO- MKT-02- 8918A

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