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Short communication

A validated reversed phase HPLC method for the determination of process-related impurities in almotriptan malate API

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Abstract

An isocratic reversed phase liquid chromatographic (RP-LC) method has been developed and subsequently validated for the determination of almotriptan malate and its process-related impurities. Separation was achieved with a Phenomenex, Gemini, C-18 column and sodium phosphate buffer (pH adjusted to 7.6): acetonitrile (80:20, v/v) as eluent, at a flow rate of 1.5 mL/min. UV detection was performed at 227 nm. The method is simple, rapid, selective, accurate and stability indicating. The described method is linear over a range of LOQ, 1.5 ug/mL (150% of the specification limit) for all the process-related impurities. The method precision for the determination of related compounds was below 1.0% R.S.D. The accuracy of the method demonstrated at 4 levels in the range of 25–150% of the specification limit and the recovery of impurities were found to be in the range of 96–102%. The method is useful in the quality control of bulk manufacturing.

Keywords: Almotriptan; Almotriptan malate; Process-related impurities; RP-LC; Validation

1. Introduction

Triptans are a family of tryptamine-based drugs used as abortive medication in the treatment of migraine and cluster headaches. While these drugs are effective at treating individual headaches, they are neither a preventative measure nor a cure. Their action is attributed to their binding to serotonin 5-HT1B and 5-HT1D receptors in cranial blood vessels (causing their constriction) and subsequent inhibition of pro-inflammatory neuropeptide release. Almotriptan malate, a selective 5-hydroxytryptamine1B/1D (5-HT1B/1D) receptor agonist, is chemically designated as 1-[[[3-[2-(dimethylamino) ethyl]-1*H*-indol-5-l]methyl]sulfonyl]pyrrolidine (±)-hydroxybutanedioate (1:1), and its empirical formula is $C_{17}H_{25}N_3O_2S$–$C_4H_6O_5$, having a molecular weight of 469.56. Almotriptan malate is a white to slightly yellow crystalline powder which is soluble in water. Almotriptan malate is used to treat severe migraine headaches. Almotriptan malate is available in market as conventional tablets (AXERT®).

Almotriptan malate was synthesized as per the process given in US5565447 [1,2]. The impurities, related to this route of synthesis, were synthesized at SMS Pharma Research Center (Hyderabad, India) and characterized. The process-related impurities are shown in Fig. 1 and the characterization data is shown in Table 1.

Till now, a few procedures based on liquid chromatography have been reported for the quantitative determination of almotriptan in human plasma and urine. Jansat et al. have published a method, using high performance liquid chromatography, for determination of almotriptan levels in plasma [3]. Fleishaker et al. have suggested a method, using a validated, sensitive and specific HPLC, for determination of almotriptan concentrations in urine [4]. So far to our knowledge, no analytical method for determination of process-related impurities in almotriptan malate API is reported in literature. So it is felt essential to develop a liquid chromatographic (LC) procedure,

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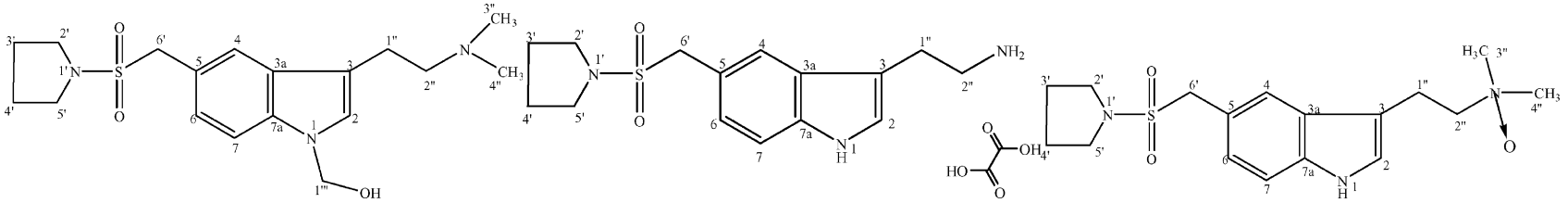
Table 1
Characterization data of synthesized impurities

Impurity name	Impurity-A	Impurity-B	Impurity-C
Structure			
^{1}H NMR	1.76/m, 4H (H-3′ and 4′), 2.68/s, 6H (H-3″ and 4″), 3.08/t, 2H (H-1″), 3.12/t, 4H (H-2′ and 5′), 3.12/t, 2H (H-2″), 4.40/s, 2H (H-6′), 5.25/s, 2H (H-1‴), 7.14/d, J = 8.5 Hz, 1H (H-6), 7.25/s, 1H(H-4), 7.35/d, J = 8.5 Hz, 1H (H-7), 7.60/s, 1H (H-2)	1.74/t, 4H (H-3′ and 4′), 2.96/t, 2H (H-1″), 3.07/t, 2H (H-2″), 3.10/t, 4H (H-2′ and 5′), 4.43/s, 2H(H-6′), 7.13/d, J = 8 Hz, 1H (H-6), 7.25/s, 1H (H-4) 7.34/d, J = 8 Hz, 1H(H-7), 7.56/s, 1H (H-2), 11.04/s, 1H (COOH)	1.74/bs, 4H (H-3′ and 4′), 2.41/s, 2H (H-1″), 3.23/s, 4H (H-2′ and 5′), 3.20/s, 2H (H-2″), 3.30/s, 6H (H-3″ and 4″), 4.50/d, J = 13 Hz; 2H (H-6′), 7.15/s, 1H (H-4), 7.20/dd, J = 8 Hz; 2Hz, 1H (H-6), 7.30/d, J = 8 Hz, 1H (H-7), 7.60/s, 1H (H-2), 7.87/s, 1H (H-1)
^{13}C NMR	21.1 (C-1″), 25.4 (C-3′ and 4′), 43.2 (C-3″ and 4″), 47.7 (C-2′ and 5′), 54.1 (C-2″), 57.8 (C-6′), 68.5 (C-1‴), 110.1 (C-7), 110.9 (C-3), 120.2 (C-6), 121.0 (C-4), 124.2 (C-5), 126.6 (C-2), 127.8 (C-3a), 135.4 (C-7a)	23.1 (C-3′ and 4′), 25.3 (C-1″), 47.6 (C-2″), 47.6 (C-2′ and 5′), 54.1 (C-6′), 109.6 (C-7), 111.3 (C-3), 119.5 (C-6), 120.6 (C-4), 124.0 (C-2), 124.0 (C-5), 126.7 (C-3a), 136.0 (C-7a), 164.8 (-COOH)	22.6 (C-1″), 25.3 (C-3′ and 4′), 47.6 (C-2′ and 5′), 50.1 (C-3″ and 4″), 54.1 (C-2″), 59.5 (C-6′), 111.5 (C-3), 112.1 (C-7), 119.3 (C-6), 120.7 (C-4), 123.7 (C-5), 124.4 (C-2), 127.1 (C-3a), 135.9 (C-7a)
Mass	366 (M + 1)	308 (M + 1)	352 (M + 1)

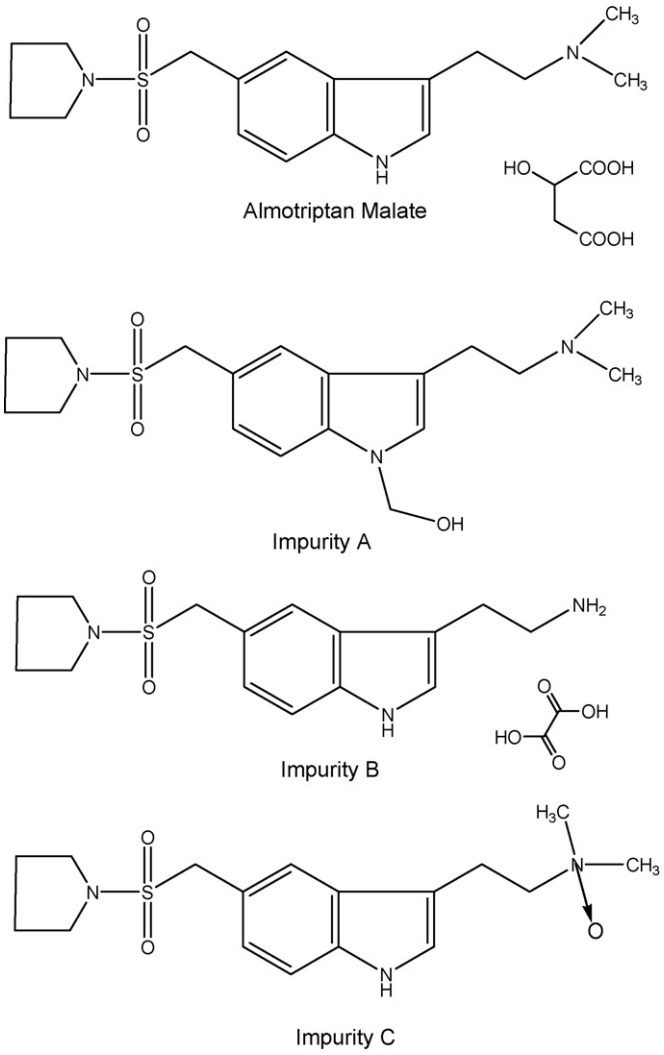


Fig. 1. Structures of almotriptan malate and its process-related impurities.

which will serve as a rapid and reliable method for the determination of process-related impurities in almotriptan malate API. In the method, developed, herein, all the process-related impurities were well separated and eluted before 12 min. The method has been thoroughly validated as per the ICH guidelines [5].

2. Experimental

2.1. Materials

Samples of almotriptan malate and their related substances were synthesized at SMS Pharma Research Centre (Hyderabad, India). HPLC grade acetonitrile was obtained from Merck (India). Analytical grade sodium dihydrogen phosphate and sodium hydroxide were purchased from SD Finechemicals (India) and malic acid (AR grade) was purchased from BDH chemicals (India). LC grade water was deionized with Milli-Q Elix and then filtered using Milli-Q Academic, Millipore water purification system (Milford, MA, USA).

2.2. Instrumentation

The LC system consisted of quaternary gradient pump, auto sampler, column oven and a photodiode array detector. The output signal was monitored and integrated using LC Solutions Chromatography Manager Software (Prominence HPLC, Shimadzu, Japan).

2.3. Solutions

2.3.1. Mobile phase

A mixture of aqueous 0.04 M sodium dihydrogen phosphate (pH of the buffer adjusted to 7.6 ± 0.1 with 10%, w/v sodium hydroxide solution) and acetonitrile in the ratio (80:20, v/v) was used as mobile phase. It was filtered through a 0.45 μ nylon membrane filter prior to use.

2.3.2. Impurity mixture

This solution was prepared using previously prepared impurity mixture (1% of each impurity spiked with respect to almotriptan malate) of all the process-related impurities at the concentration of 1 mg/mL using mobile phase and injected into the system

2.3.3. Standard solution

0.001 mg/mL solution of almotriptan malate, working standard, was prepared using mobile phase and injected into the system.

2.3.4. Sample solution

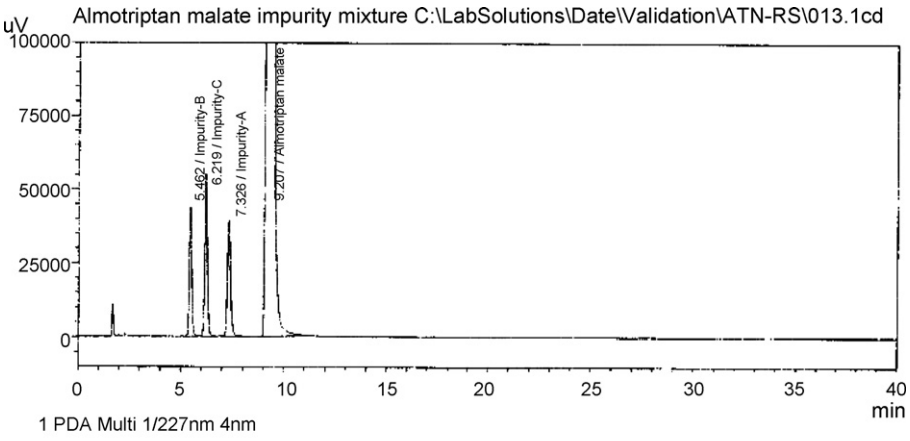
One milligram per milliliter solution of almotriptan malate sample was prepared using mobile phase and injected into the system.

2.3.5. Forced degradation samples for specificity study

Almotriptan malate was heated with aqueous 5N hydrochloric acid solution at 80 °C for 3 h and separately with aqueous 5N sodium hydroxide at 80 °C for 4 h to study formation of degradation products under acidic and basic conditions, respectively. Almotriptan malate sample was heated with 5% hydrogen peroxide solution at 80 °C for 30 min to study formation of degradation products under oxidative condition. To study degradation products under photolytic and thermal degradations, almotriptan malate sample was exposed to ultraviolet light (254 nm) for 12 h and another sample was kept at 105 °C temperature for 12 h, respectively.

2.4. Conditions

A Gemini C18 analytical column (250 mm × 4.6 mm, 5 μ packing) (Phenomenex) was used for analysis at 30 °C. The mobile phase was pumped through the column at a flow rate of 1.5 mL/min. The sample injection volume was 10 μL. The photodiode array detector was set to a wavelength of 227 nm for the detection.



Peak table

PDA Ch1 227nm 4nm

Peak#	Ret.Time	Resolution	Relative Retention Time	Name
1	5.462	0.0	0.59	Impurity-B
2	6.219	3.2	0.68	Impurity-C
3	7.326	4.1	0.80	Impurity-A
4	9.207	5.3	1.00	Almotriptan malate
Total				

Fig. 2. System suitability chromatogram of almotriptan malate.

3. Results and discussion

3.1. Method development

3.1.1. Separation of process of process-related impurities

In order to develop a suitable and robust LC method for the determination of almotriptan malate and its process-related impurities, different mobile phases and columns were employed to achieve the best separation and resolution. Finally, the mobile phase consisting of aqueous 0.04 M sodium dihydrogen phosphate with a pH 7.6 and acetonitrile in the ratio of 80:20 (v/v) at a flow rate of 1.5 mL/min using Gemini C18, 250 mm column was found to be appropriate, allowing good separation of almotriptan and its process-related impurities. The peaks are very symmetrical. In the above conditions malic acid eluted first followed by impurities B, C and A. In the present method the selectivity was found to be more than 1.1 with a resolution greater than 3.2 for all the compounds. System suitability results of the method are presented in Fig. 2. The chromatograms showing the separation of all the degradation products are shown in Fig. 3. Almotriptan malate and its process-related impurities show significant UV absorbance at wavelength 227 nm. Hence, this wavelength has been chosen for detection in the analysis of almotriptan malate.

Table 2
Parameters of linearity curve of impurities of almotriptan malate

Impurity name	Slope	Y-intercept	Correlation coefficient
Impurity A	43407.4	28.4	0.9999
Impurity B	38995.2	34.5	0.9999
Impurity C	43192.2	−2.4	0.9999

3.1.2. Quantification of process-related impurities

The relative response factors (RRF) of impurities A, B & C with respect to almotriptan malate were found to be 1.2, 1.0 and 1.2, respectively, rounded off to 1.0 since all are between 0.8 and 1.2. The weight percentage of the impurity present in almotriptan malate sample was calculated using its RRF value and peak response.

3.2. Method validation

The LC method developed has been extensively validated for the process-related impurities of almotriptan malate using the following parameters. Standard solution was used for the purpose of quantification of process-related impurities in almotriptan malate.

Table 3
Parameters of recovery of impurities of almotriptan malate

Impurity name	Spike level (%)	Added (μg)	Recovered (μg)	% Recovery
Impurity-A	25	6.87	7.01	102
	50	13.73	14.0	102
	100	27.46	27.57	100
	150	41.19	40.91	99
Impurity-B	25	6.14	5.94	97
	50	13.73	13.97	102
	100	27.46	27.57	100
	150	41.19	40.91	99
Impurity-C	25	6.46	6.47	100
	50	13.73	13.97	102
	100	27.46	27.57	100
	150	41.19	40.91	99

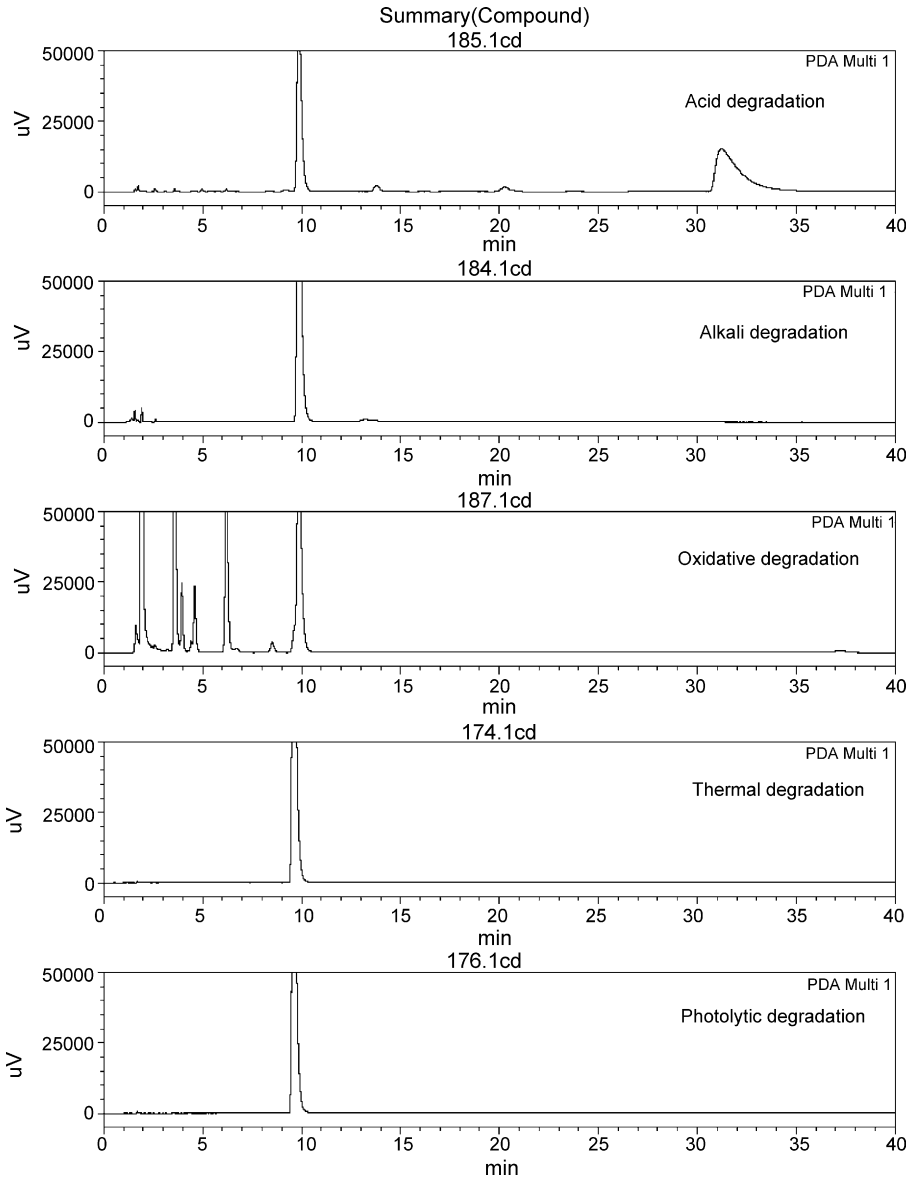


Fig. 3. Forced degradation chromatograms of almotriptan malate.

3.2.1. Specificity

The specificity of the method was demonstrated by adding all the possible process-related impurities, discussed above, to pure almotriptan malate sample and analyzed. Forced degradation studies were performed to demonstrate the validity of the method. The sample treated with sodium hydroxide did not cause any degradation and similarly the sample exposed to thermal condition as well as the one exposed to UV light not lead to any traceable degradation. But the sample heated with 5N HCl and 5% H_2O_2 were mostly converted to degraded products and these are well separated from almotriptan peak. Photodiode array detection was also used as evidence of the specificity of the method, and to evaluate the homogeneity of the peak. The samples exposed to acidic, basic, oxidative, thermal and UV stress conditions were subjected to photodiode array analysis for peak purity of almotriptan. The plots with flat tops in all instances showed that almotriptan 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 is specific and able to resolve all the process-related impurities and degradation products and can be used for determining the stability of almotriptan malate in bulk. A typical chromatogram of the impurities in the real sample and the same sample spiked with impurities are shown in Fig. 4.

3.2.2. Linearity

Standard solutions at nine different concentration levels ranging from LOQ to 0.15 μg/mL (150% of specification limit) were prepared and analyzed in duplicate in order to demonstrate the linearity for all the impurities. Linearity regression analysis demonstrated acceptability of the method for quantitative determination range of LOQ to 150% of the specification limit. The coefficient of correlation was found to be more than 0.999. The

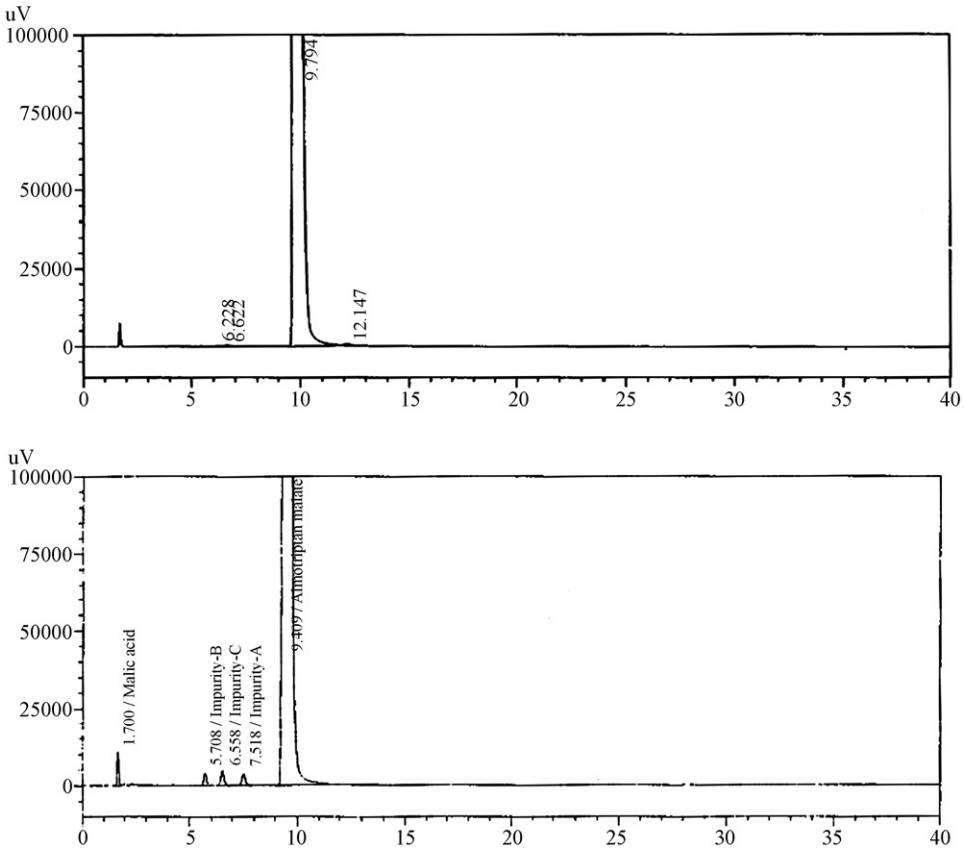


Fig. 4. Typical chromatogram of the impurities in the real sample of almotriptan malate.

values of slope, intercept and coefficient correlation for each impurity are shown in Table 2.

3.2.3. Accuracy

Accuracy of the method was demonstrated at four different concentration levels in triplicate. The analysis carried out at 25%, 50%, 100% and 150% of specification limit. The mean recoveries of all the impurities were found to be in the range of 96–102% as shown in Table 3.

3.2.4. Precision

The precision of the method for the determination of impurities related to almotriptan malate, was studied for repeatability and intermediate precision. Repeatability was demonstrated by analyzing almotriptan malate sample six times. Intermediate precision was demonstrated by analyzing same sample of almotriptan malate by two different chemists on two different days. Intra-day variations of impurities of amotriptan malate are expressed in terms of %R.S.D. values. Repeatability and intermediate precision for the process-related impurities in almotriptan malate were found to be <10.0%R.S.D.

3.2.5. Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection and limit of quantification for impurities A–C were calculated from the linearity data using residual standard deviation of the response and slope of the calibration curve for each impurity. The limit of detection of a compound is defined as the lowest concentration that can be detected. LOD values were found to be 0.024, 0.022 and 0.022 ug/mL for impurity A, B and C, respectively. The limit of quantification is the lowest concentration of a compound that can be quantified with acceptable precision and accuracy. LOQ values were found to be 0.079, 0.075 and 0.075 ug/mL for impurity A, B and C, respectively.

3.2.6. Robustness

In order to demonstrate the robustness of the method, system suitability parameters were verified by making deliberate changes in the chromatographic conditions, viz. change in flow rate by ±0.1 mL/min, change in pH of the buffer ±0.1 unit and change in the ratio of mobile phase (±2% absolute). The method was demonstrated to be robust over an acceptable working range of its HPLC operational parameters.

4. Conclusion

The present paper describes the development of a new HPLC method for the determination of process-related impurities in almotriptan malate API and its validation. The method was found to be selective, sensitive, precise and accurate for the determination of process-related impurities and degradation products.

This method can be used for the routine determinations in pharmaceutical quality control.

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