DEVELOPMENT OF METHOD FOR DETERMINATION OF DIACEREIN IN RAW MATERIAL BY HPLC

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SUMMARY

An HPLC-DAD method was developed and validated for determination of diacerein in raw material. The chromatographic conditions are as follows: Column: Luna RP-C18 (4,6 mm x 250 mm; 5 μm). Detector UV, λ = 272 nm. Mobile phase: Buffer solution : Acetonitrilee (55 : 45), (Buffer solution: Mix 19.6 mL of 0.1 mol/L Citric acid and 0.4 mL of 0.2 mol/L sodium monohydrogen phosphate); Flow rate: 1.5 ml/min. Temperature: room temperature.

Keywords: *Diacerein, HPLC, method validation, quantititation*

Introduction

Drug products containing diacerein have been licensed in France since 1992 under the brand names ART 50® and Zondar® for the symptomatic treatment of osteoarthritis. Diacerein shows its anti-inflammatory activity by inhibiting the action of interleukin-1, a protein involved in inflammation and playing an important role in joint diseases[1]. Drug products containing diacerein are taken orally and are marketed in several European countries such as Austria, Czech Republic, France, Greece, Italy, Portugal, Slovakia and Spain. In Vietnam, the Drug Administration of Vietnam also grants permission for many brand-name drugs manufactured in Europe and Korea. Currently, the common international and domestic pharmacopoeias do not have monographs on diacerein [2, 3, 4]. This study is conducted to provide a reference method to help domestic drug manufacturers check the quality of input materials with available conditions at the facility.

Materials and analytical method

Instruments

Instruments have been calibrated according to ISO/IEC 17025 and GLP includes:

- HPLC model Agilent 1200, Shimadzu LC-20A
- Analytic scale Sartorius BSA 224S
- Glassware.

Reagents and solvent

- Reference standard supplied by Cassel Research Laboratories PVT. LTD (India), Assay 99.86%, Lot No.: CA/WS/200.
- Solvents for HPLC: acetonitrile, N, N-Dimethylacetamide.

Methodology

Chromatographic condition

- Column Luna RP-C18 (4.6 mm x 250 mm; 5 µm)

- Detector: UV, λ = 272 nm.

- Flow rate: 1.5 ml/min.

- Injection volume: 20 µl.

- Column temperature: room temperature

- Mobile phase: Buffer solution : Acetonitrile (55 : 45)

Preparation of buffer solution: Mix 19.6 mL of Citric acid 0.1M and 0.4 mL of Na_2HPO_4 0.2 M

Analytical method

- *Standard solution:* Transfer about 50 mg of Diacerein RS, weigh accurately, into a 50-mL volumetric flask. Dissolve and make to volume with N, N-Dimethylacetamide, mix well. Take 2.0 ml of this solution into a 50-mL volumetric flask, add mobile phase to volume.

- *Test solution:* Weigh accurately 50 mg of the sample, into a 50-mL volumetric flask. Dissolve and make to volume with N, N-Dimethylacetamide, mix well. Take 2.0 ml of this solution into a 50-mL volumetric flask, add mobile phase to volume.

- *Procedure:* Separately inject equal volumes (about 20 µl) of the standard solution and the test solution into HPLC system, record the chromatogram for all injections, record peak response of major peak.

- *Calculation:* Percentage of diacerein in the raw material is taken:

$$\frac{R_u}{R_s} \times \frac{W_s}{50 \times 25} \times \frac{50 \times 25}{W_u} \times \frac{\% P}{100} \times 100$$

In which: Ru: peak response of diacerein from the test solution (mAU.s); Rs: peak response of diacerein from the standard solution (mAU.s), Ws: weight of diacerein RS used for the standard solution (mg); Wu: weight of sample used for the test solution (mg); % P: assay of the reference standard.

Method validation: Performed according to the ASEAN guideline for method validation.

Results

System suitability

Six repeated injections of the standard solution of diacerein prepared above (concentration about 0.4 mg/ml). Results are shown in Table 1.

Bảng 1. The results of System suitability

No.	Retention time (minutes)	Peak area (mAU.s)
1	4.515	1185.7

2	4.518	1184.2
3	4.522	1178.9
4	4.509	1186.1
5	4.513	1185.3
6	4.526	1180.7
Mean	**4.517**	**1183.5**
RSD (%)	**0.14**	**0.25**
Theoretical plates: 3375		
Tailing factor: 1.91		

Specificity

With selected method, separately inject following solutions into HPLC system:

- Blank solution: Mobile phase

- Test solution: *Test solution* used in Precision

- Standard solution: *Standard solution* used in Precision

Separately inject the blank, the test solution, the standard solution into HPLC system. Record the chromatograms of all injections.

Linearity

Preparation of standard solutions at concentrations of diacerein from 20.32 µg/ml to 60.40 µg/ml, conducting chromatographic with above condition. Results are presented in Table 2.

Table 2. *The results of linearity of the method*

% Level	Weight of diacerein (mg)	Concentration of diacerein (µg/ml)	Peak response of diacerein (mAU.s)
50%	25.4	20.32	593.8
80%	40.1	32.08	941.5
100%	50.3	40.24	1183.5
120%	60.3	48.24	1412.6
150%	75.5	60.40	1783.4
Slope	29.608		
Intercept	- 8.9403		
Correlation coefficient	0.9999		

$y = 29.608x - 8.9403$
$R^2 = 0.9999$
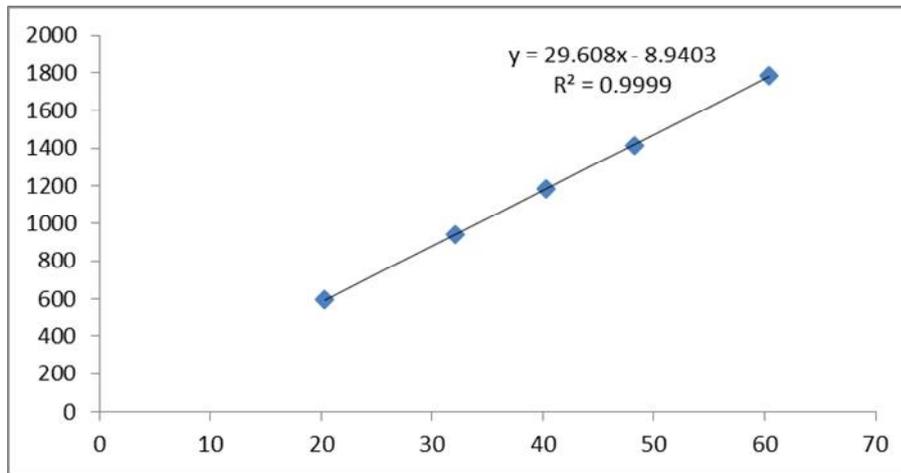
Precision

Determined by Method precision and Intermediate precision.

Method precision

Preparation of 6 samples (the Test solution) as described in the *Analytical Method.* Separately chromatograph the Test solutions. Results are presented in Table 3.

Table 3. *The results of Method precision*

No.	Weight of sample (mg)	Peak area of Diacerein in test solution (mAU.s)	Result (%)
1	50.2	1180.5	99.7
2	50.5	1182.3	99.3
3	50.7	1194.6	99.9
4	49.6	1165.2	99.6
5	49.2	1157.3	99.7
6	48,6	1141.9	99.6
Weight of Diacerein RS (mg) = 50.3 Peak area of Diacerein in standard solution (mAU.s) = 1183.5			
Mean			**99.6**
RSD (%)			**0.21**

Intermediate precision

Intermediate precision of the method is determined by analyzing six samples (test solution) as described in *Analytical Method* by two different analysts at different days. Results are presented in Table 4.

Table 4. *The results of Intermediate precision*

No.	Analysist 1 Weight of Diacerein RS (mg) = 50.3	Analysist 2 Weight of Diacerein RS (mg) = 50.5

	Peak area of Diacerein in standard solution (mAU.s) = 1183.5		Peak area of Diacerein in standard solution (mAU.s) = 1191.8	
	Peak area of Diacerein in test solution (mAU.s)	Result (%)	Peak area of Diacerein in test solution (mAU.s)	Result (%)
1	1180.5	99.7	1172.7	99.1
2	1182.3	99.3	1164.6	99.4
3	1194.6	99.9	1170.3	99.3
4	1165.2	99.6	1196.9	100.6
5	1157.3	99.7	1191.4	100.5
6	1141.9	99.6	1194.2	99.4
	Mean: 99.6% **RSD: 0.21%**		**Mean: 99.7%** **RSD: 0.64%**	
	Mean: 99.7% **RSD: 0.46%**			

Accuracy

Accuracy of the assay test of diacerein in raw material was assessed by percentage recovered of known assay diacerein RS. Preparation of 9 samples, concentration of each 3 samples were equivalent to 80%, 100%, 120% of the nominal assay concentration:

- Transfer accurately quantities of diacerein standard, equivalent to about 40 mg, 50 mg, 60 mg of diacerein, to 50 ml volumetric flasks. Add 30 ml of N, N-Dimethylacetamide, ultrasonicate and make to volume with N, N-Dimethylacetamide, filter. Take 2.0 ml of these filtrates, into 50-mL volumetric flasks and make to volume with mobile phase. Results are presented in Table 5.

Table 5. *Results for Accuracy*

Sample	Weight of diacerein RS (mg)	Peak area of diacerein	Diacerein recovered (mg)	Percentage recovered (%)
80%	40.3	986.1	40.5	100.4
80%	40.5	980.3	40.2	99.3
80%	40.7	982.5	40.3	99.0
			Mean	**99.6**
			RSD (%)	**0.72**
100%	50.0	1208.4	49.6	99.2
100%	50.2	1203.7	49.4	98.4

100%	50.6	1230.9	50.5	99.8
			Mean	**99.1**
			RSD (%)	**0.72**
120%	60.1	1452.3	59.6	99.1
120%	60.4	1461.2	60.0	99.3
120%	60.8	1467.5	60.2	99.0
			Mean	**99.1**
			RSD (%)	**0.11**
Mean				*99.3*
RSD (%)				*0.56*

Discuss

Results for System suitability showed Theoretical plates N = 3375, Tailing factor R = 1.91. Therefore, chromatographic system is suitable for determination of diacerein in raw material. The retention time of diacerein peak from the *Test solution* chromatogram is about 4.5 minutes, the retention time of diacerein peak from the *Standard solution* chromatogram is about 4.5 minutes. There is no peak in the blank solution at or about the retention time of diacerein. Therefore, the method is specific for determination of diacerein in raw material. For linearity, the analytical method for determination of diacerein in raw material shows linearity across the concentrations from 20.32 µg/ml to 60.40 µg/ml. The method has high precision with RSD = 0.21% and the intermediate precision is good with RSD = 0.64%. Therefore, the method does meet the requirement for precision. Average recovery is 99.3% in the range of 80% to 120% of the nominal content with RSD = 0.56%.

Conclusion

The study has developed and validated a method to determine diacerein in raw materials by HPLC. The experimental results show that the selected chromatographic conditions (column C18, mobile phase: buffer - acetonitrilee (55 : 45), flow rate 1.5 ml/min, injection volume 20 µl) are suitable for determination of diacerein in raw materials.

References

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