

RP-HPLC Method for Estimation of Ado-Trastuzumabe Emtansine Injection in Pharmaceutical Dosage Form

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The aim of the present work is to develop and validate a simple, reliable and accurate method for ado-trastuzumab emtansine in pharmaceutical dosage form by reverse phase HPLC method. The proposed RP-HPLC (reverse phase-high performance liquid chromatography) method utilized a WATERS HPLC (model-Agilent 2695), C18 RP Column (150 mm × 4.6 mm × 5 µm) in separation mode with mobile phase consisting of 0.1 M potassium dihydrogen phosphate (KH₂PO₄) and methanol in the ratio of 55: 45 % (v/v), at a flow rate 1.1 mL/min and the effluent was monitored at 242 nm. The retention time of ado-trastuzumabe emtansine was 3.2 min. The method was linear in range of 50 to 150 µg and the correlation coefficient was 0.999. The percentage recovery was 100 %. LOD (limit of detection) and LOQ (limit of quantitation) for this method were found to be 0.236 and 0.787, respectively. The present method is very simple, sensitive and accurate procedure for estimation of ado-trastuzumabe emtansine.

Keywords: Ado-trastuzumabe emtansine, Reverse phase-high performance liquid chromatography, Validation.

INTRODUCTION

The antibody-drug compound ado-trastuzumab emtansine (T-DM1) is integrate the anticancer substances which is a potent microtubule-disrupting drug and linked by a stable coupler of the humanized anti-human epidermal growth factor receptor 2 (HER2) antibody, trastuzumab, with the maytansinoid, DM1 [1-3]. Therefore attached to HER2, the compound is internalized *via* receptor-mediated endocytosis and an active derivative of DM1 is eventually delivered by proteolytic degradation of the antibody moiety within the lysosome [1,4]. HER 2-positive and metastatic breast carcinoma treatment is possible by this agent who have *hitherto* used taxane or trastuzumab. Ado-trastuzumab emtansine has a lengthy biological half-life of about 4 days [5].

The objective of the present study is to develop and validate a HPLC method for ado-trastuzumab emtansine pharmaceutical dosage form to be indenture in routine analysis [6]. In the method development of ado-trastuzumab emtansine we have incorporating the reverse phase high performance liquid chromatography (HPLC). Then the developed method will be validated according to ICH guidelines for its various parameters [7-9].

EXPERIMENTAL

Ado-trastuzumab emtansine was obtained as a gift sample from Hetero Labs Limited, Hyderabad, Telangana, India. HPLC grade methanol and water were purchased from Merck, India and Loba Chemie Pvt. Ltd., India, respectively. All the other chemicals used were of analytical grade purchased from Finar chemicals, India.

HPLC instrumentation and conditions: Quantitative HPLC was performed on WATERS HPLC, model: Agilent 2695, photo diode detector (PDA), with an automated sample injector. The output signal was monitored and integrated using Empower 2 software. Phenomenex C18 (150 mm × 4.6 mm × 5 µm) column was used for separation, maintained at 25 °C, eluted with mobile phase at a flow rate of 1.1 mL/min for 10 min. The mobile phase consisted of 0.1 M potassium dihydrogen phosphate (KH₂PO₄) and methanol in the proportion of 55: 45 % (v/v). Measurements were made with injection volume 8 µL and UV detection at 242 nm.

Preparation of mobile phase: 0.1 M Potassium dihydrogen phosphate (13.608 g of KH₂PO₄ was added to 1000 mL of HPLC water to make 0.1 M solution of KH₂PO₄) and methanol were mixed properly in the ratio of 55:45.

Preparation of standard and sample solutions

Standard solution: The standard stock solution of ado-trastuzumab emtansine (1 mg/mL) was prepared by dissolving 100 mg of ado-trastuzumab emtansine in 100 mL volumetric flask containing 20 mL of methanol and sonicated for 10 min and made up with water. From the above solution, 2.5 mL was transferred into a 25 mL volumetric flask and diluted up to the mark with water.

Sample solutions: 5 mL of commercially available pharmaceutical dosage form of Ado-Trastuzumab emtansine (Kadcyla Injection) equivalent to 100 mg of ado-trastuzumab emtansine was transferred into a 100 mL volumetric flask and 20 mL of methanol was added and sonicated for 20 min and made up with water. From the above solution, 2.5 mL was transferred into a 25 mL volumetric flask and diluted up to the mark with methanol and solution was filtered through 0.45 μ m filter before injecting into HPLC system.

RESULTS AND DISCUSSION

Optimization of the method: The RP-HPLC method utilized Phenomenex C18 column (150 mm $\times$ 4.6 mm $\times$ 5 μ m) in an isocratic separation mode with mobile phase 0.1 M potassium dihydrogen phosphate and methanol in the proportion of 55:45 % (v/v), at a flow rate of 1.1 mL/min and the effluent was monitored at 242 nm. The retention time for ado-trastuzumab emtansine was 3.234 min (Fig. 1).

Method validation: The method has been validated for system suitability, specificity, accuracy, precision, linearity, robustness, limit of detection (LOD) and limit of quantitation (LOQ).

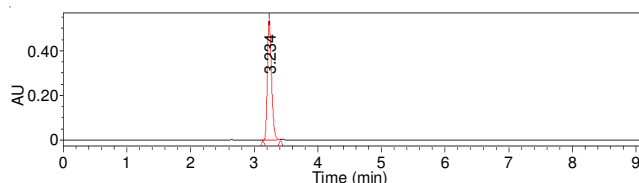


Fig. 1. Chromatogram for optimized method of ado-trastuzumab emtansine

System suitability: The system suitability parameters like retention time, theoretical plates, tailing factor were calculated and compared with the standard values whether the proposed RP-HPLC method for the estimation of ado-trastuzumab emtansine in pharmaceutical formulations was validated or not. The obtained value of retention time in this method was 3.224 min, theoretical plates were 10783 and tailing factor was found to be 1.28.

Specificity: The time (R_t) for standard, sample and commercial product of ado-trastuzumab emtansine were same (R_t = 3.22 min). This proved that excipients have no effect on the analytical method.

Accuracy: The present study was performed at three levels in which sample stock solution was spiked with standard drug solution containing 50, 100 and 150 % of labeled amount of drugs. Three replicate samples of each concentration level were prepared and the % recovery at each level ($n = 3$) and mean % recovery ($n = 9$) were determined (Table-1 and Fig. 2). The mean recovery was 100 %.

Precision: The precision of the proposed method was evaluated by carrying out six independent assays of test sample. Percentage relative standard deviation (% RSD) was found to be less than 2 % which proved that method was precise (Table-2 and Fig. 3).

TABLE-1
ACCURACY (% RECOVERY) RESULTS OF ADO-TRASTUZUMAB EMTANSINE

Accuracy level (%)	Sample name	Sample weight	Added (μ g/mL)	Found (μ g/mL)	Recovery (%)	Mean (%)
50	1	50.00	49.500	49.67	100	100
	2	50.00	49.500	49.52	100	
	3	50.00	49.500	49.72	100	
100	1	100.00	99.000	99.16	100	100
	2	100.00	99.000	99.42	100	
	3	100.00	99.000	99.27	100	
150	1	150.00	148.500	149.21	100	100
	2	150.00	148.500	149.23	100	
	3	150.00	148.500	149.29	101	

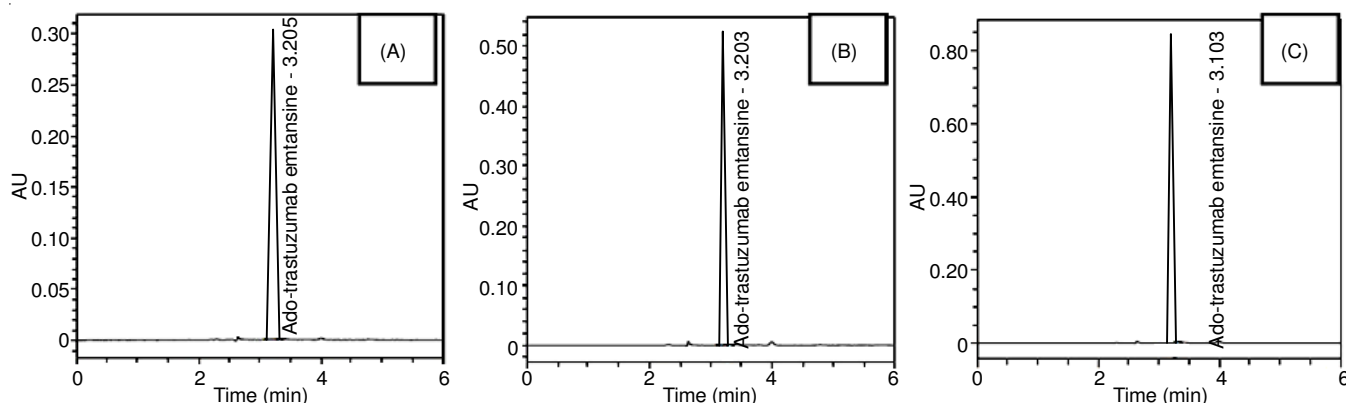


Fig. 2. Typical chromatogram for accuracy 50 % (A), accuracy 100 % (B) and accuracy 150 % (C)

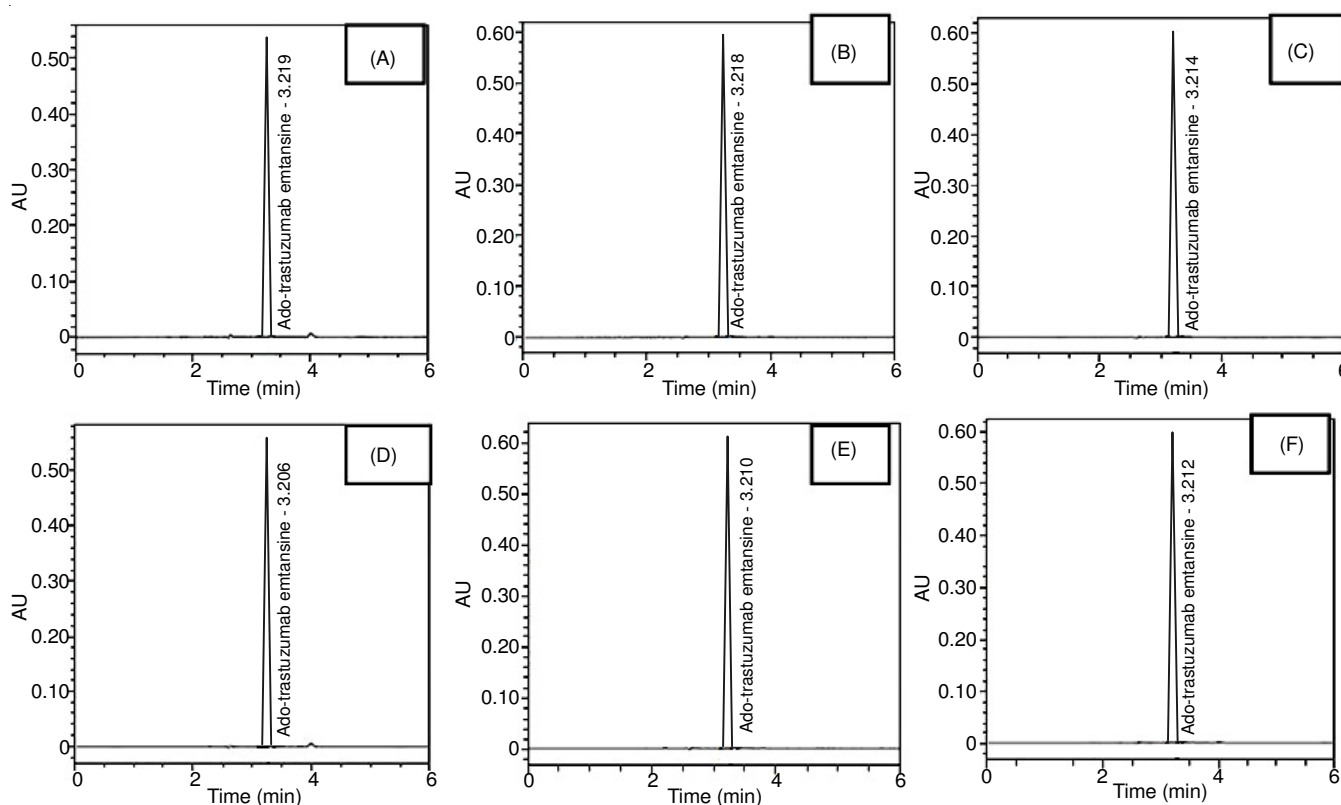


Fig. 3. Chromatogram for precision injection 1 (A), injection 2 (B), injection 3 (C), injection 4 (D), injection 5 (E) and injection 6 (F)

TABLE-2

PRECISION DATA FOR ADO-TRASTUZUMAB EMTANSINE

S. No.	R_t	Area	Assay (%)
Injection 1	3.219	2460978	99
Injection 2	3.218	2465404	99
Injection 3	3.214	2461769	99
Injection 4	3.206	2460257	99
Injection 5	3.210	2461567	99
Injection 6	3.212	2466050	99
Mean			99
Std. Dev.			0.10
RSD (%)			0.10

TABLE-3

ROBUSTNESS DATA FOR ADO-TRASTUZUMAB EMTANSINE

Parameter	R_t	Theoretical plates	Asymmetry
Decreased flow rate (0.8 mL/min)	3.920	13834	1.17
Increased flow rate (1.2 mL/min)	2.726	11801	1.17
Decreased temperature (20 °C)	3.208	12638	1.16
Increased temperature (30 °C)	3.205	12746	1.15

Linearity: A linear relationship between peak areas *versus* concentrations was observed for ado-trastuzumab emtansine in the range of 50 to 150 µg/mL of nominal concentration. Correlation coefficient was 0.999 for ado-trastuzumab emtansine which proved that the method was linear in the range of 50 to 150 µg/mL.

Robustness: The results of robustness of the present method had shown that the changes made in the flow and temperature did not produce significant changes in analytical results which were presented in Table-3 and Fig. 4. So, the method conditions were robust.

LOD and LOQ: Minimum concentration of standard component in which the peak of the standard gets merged with noise is called as LOD (limit of detection) and minimum concentration of standard component in which the peak of the standard gets detected and qualified is called as LOQ (limit of quantitation). The formulae used were $LOD = 3.3 \sigma/S$ and $LOQ = 10 \sigma/S$ (where σ = standard deviation of response and S =

slope of calibration curve). LOD and LOQ for this method were found to be 0.236 and 0.787, respectively.

Conclusion

The present study is focused to develop and validate HPLC method for estimation of ado-trastuzumab emtansine in pharmaceutical dosage form. For routine analytical purpose it is desirable to establish methods capable of analyzing huge number of samples in a short time period with good robustness, accuracy and precision without any prior separation steps. HPLC method generates a large amount of quality data which serve as highly powerful and convenient analytical tool. The method showed good reproducibility and good recovery. From the specificity studies, it was found that the developed method was specific for ado-trastuzumab emtansine which was related to all the parameters of the methods.

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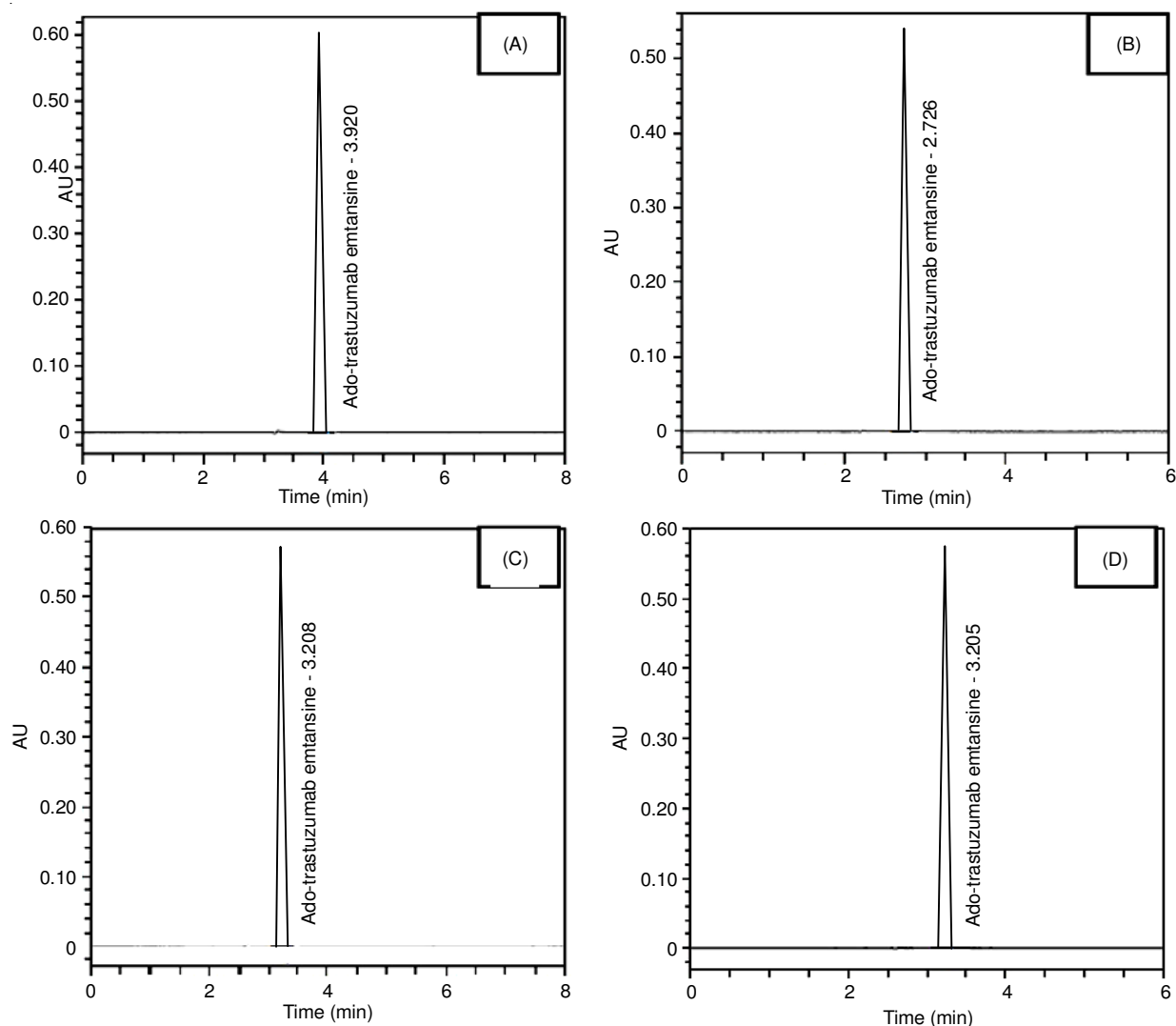


Fig. 4. Chromatogram for decreased flow rate (A), increased flow rate (B), decreased temperature (C) and increased temperature (D)

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