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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD TO SEPARATE LOW LEVELS OF ATOLTIVIMAB, MAFTIVIMAB AND ODESIVIMAB AND OTHER RELATED COMPOUNDS

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ABSTRACT
A method for estimation of drug components of InmazebTM injection using RP-HPLC method is reported in this article. Quantitative estimation of Atoltivimab (ATO), Maftivimab (MAF), and Odesivimab (ODE) and validation of the proposed method as per the ICH guidelines is reported in this article. Forced degradation studies were carried out to study the interference of degradation products in the estimation of drug components and are found to be within regulated limits.

KEYWORDS: Atoltivimab, Maftivimab, Odesivimab, ICH guidelines, Quantification.

1. INTRODUCTION

Maftivimab (MAF), Atoltivimab (ATO), and odesivimab (ODE) are Monoclonal antibodies MAF, ATO and ODE are combined in Inmazeb™.[1-3] Monoclonal antibodies have been employed to manage a variety of illnesses, notably cancer.[4-6] As these antibodies attempt to locate, bind to, and assault a particular target on something like a cancer cell, certain monoclonal antibodies employed to fight cancer are considered as the targeted therapy.[7-10] Inmazeb™ (MAF, ATO & ODE) has been used to treat Zaire ebolavirus infection in children & adults, notably neonates born to a woman who has tested positive for Zaire ebolavirus.[11-16] Despite this clinical significance, no analytical approach for combinational assay of atoltivimab (ATO), maftivimab (MAF), and odesivimab (ODE) has been reported in the existing literature. So, Authors proposed a method for simultaneous estimation of the drug components of Inmazeb™ by reverse phase HPLC method without interference of additives of the formulation.

2. EXPERIMENTAL

2.1. Material and chemicals

Atoltivimab (ATO), Maftivimab (MAF) and Odesivimab (ODE) pure active pharmaceutical ingradients were procured from Sun Life Sciences Limited and Inmazeb™ injection purchased from local pharmacy store, Phosphoric acid & Hydrochloric acid (**Source** - SD Fine-Chem limited, India), Water (HPLC grade), Sodium Hydroxide, Sodium Dihydrogen Phosphate(**Source** - Ven Life Sciences Pvt. Ltd, India) , Acetonitrile(**Source** - Merck specialties limited, India.)

2.2. Instrumentation and RP-HPLC conditions

2.2.1. Instrumentation

RP-HPLC system (**Model** - 2695 Model, **Description** - Water alliance), Column-Sunsil C_{18} - 250 mm×4.6 mm, 5µm**,** Software (**Model** - Empower, **Description** -.Water alliance), Photodiode array detector (**Model** -2998 Model, **Description** - Water alliance).

2.2.2. Optimized HPLC Conditions

Flow rate was adjusted to 1.0 ml/min, Injection volume was adjusted to10µl, Temperature was maintained at 25°C, Detector wavelength was adjusted to 267 nm and Run time total was 10 min. **Mobile phase:** NaH_2PO_4 (strength of 0.1M & pH 3.5) and acetonitrile are mixed in the ratio 40:60 vol/vol.

2.3. Stock of ATO, MAF & ODE solution: This was prepared with ATO quantity of 166.70 µg/ml, MAF quantity of 166.70 µg/ml and ODE quantity of 166.70 µg/ml by dissolving 16.67 mg of ATO, 16.67 mg of MAF and 16.67 mg of ODE 100 ml of dissolving solvent [NaH_2PO_4, strength of 0.1M & pH 3.5 and acetonitrile (pure) are mix in the ratio 40:60 vol/vol fraction].

2.4. Working of ATO, MAF & ODE solutions: 5 ml stock ATO (166.70 µg/ml), MAF (166.70 µg/ml), & ODE (166.70 µg/ml) mixed with 45 ml dissolving solvent [NaH_2PO_4, strength of 0.1M & pH 3.5 and acetonitrile are merged in the ratio 40:60 vol/vol].

2.5. ATO, MAF & ODE Calibration curves

Five different concentrations of ATO, MAF, & ODE calibration solutions had prepared with varying concentrations of ATO (8.335 – 25.005 µg/ml), MAF (8.335 – 25.005 µg/ml), & ODE (8.335 – 25.005 µg/ml).The peak responses related to ATO, MAF, & ODE were recorded by using optimized chromatographic conditions. Calibration curves were plotted for ATO, MAF, & ODE.

2.6. INMAZEB ™ ATO, MAF & ODE solution

The stock Inmazeb™ solution was prepared with ATO quantity of 166.70 µg/ml, MAF quantity of 166.70 µg/ml and ODE quantity of 166.70 µg/ml by dissolving Inmazeb™ powder having 16.67 mg of ATO, 16.67 mg of MAF and 16.67 mg of ODE in 100 ml of diluent and sonicated, filtered through membrane filter.

Working Inmazeb™ solution: 5 ml stock Inmazeb™ solution having ATO (166.70 µg/ml), MAF (166.70 µg/ml), & ODE (166.70 µg/ml) mixed with 45 ml of diluent.

2.7. Analysis of ATO, MAF & ODE IN INMAZEB™ formulation:

At optimized chromatographic conditions, the peak responses related to ATO, MAF, & ODE were recorded and the contents were calculated from the peak areas.

2.8. Stress studies

The stock Inmazeb™ solution with ATO quantity of 166.70 µg/ml, MAF quantity of 166.70 µg/ml and ODE quantity of 166.70 µg/ml was stressed consistent with ICH directives[23] with conditions such as acid hydrolysis, oxidation by peroxide, base hydrolysis, degradation through dry heat, Degradation in sunlight and the chromatograms were recorded by using optimized chromatographic conditions. The degradation of ATO, MAF, and ODE were evaluated in Inmazeb™ formulation injection based on peak responses of ATO, MAF & ODE.

3. RESULTS AND DISCUSSION

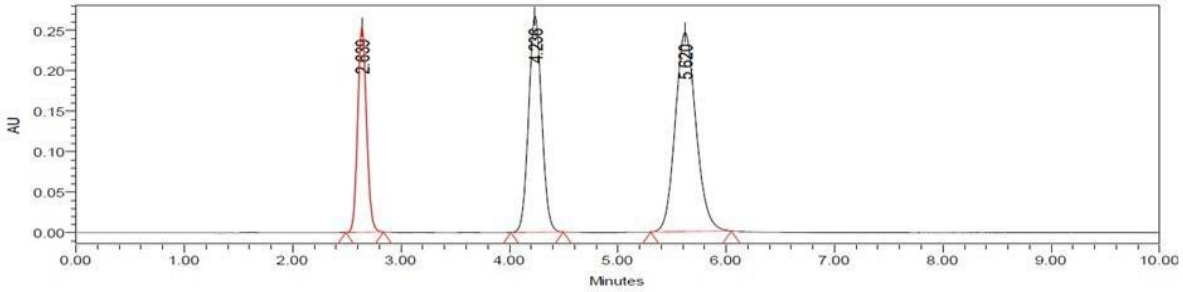

Fig.1: Measurements and chromatograms.

Table.1: Chromatographic Specifications

Name	Retention Time	Area	% Area	Height	USP Resolution	USP Tailing	USP plate count
ATO	2.639	1445493	20.74	253138	-	1.10	4946
MAF	4.236	2242891	32.18	266724	8.46	1.05	5798
ODE	5.620	3282322	47.09	245861	4.76	1.13	4163

3.1. Validation

3.1.1. Linearity

ATO, MAF, & ODE calibration curves have plotted. Five concentration points were used to plot the ATO, MAF, & ODE curves. The least-square strategy indicates that significant linear calibrating fit for ATO in the range of 8.335 – 25.005 µg/ml, for MAF in the range 8.335 – 25.005 µg/ml and for ODE in the range of 8.335 – 25.005 µg/ml.

Table 2: Linearity data.

ATO		MAF		ODE	
µg/ml	Area	µg/ml	Area	µg/ml	Area
8.335	909608	8.335	1394853	8.335	2006718
12.50	1344779	12.50	2093802	12.50	3023367
16.67	1801377	16.67	2793535	16.67	4030849
20.8375	2252932	20.8375	3495605	20.8375	5042730
25.005	2716826	25.005	4201276	25.005	6069487

3.1.2.LOD

From the regression line of ATO, MAF, and ODE , slope and standard deviation, Limit of detection is calculated by using the following formula.

$$\text{Limit of detection} = 3.3 \times \frac{\text{standard deviation of area value}}{\text{linearity curve slope value}}$$

- LOD: ATO – 0.059 µg/ml; S/N level ratio - 3.4
- LOD: MAF – 0.054 µg/ml; S/N level ratio - 3.7
- LOD: ODE – 0.059 µg/ml; S/N level ratio - 3.2

3.1.3.LOQ

From the regression line of ATO, MAF, and ODE , slope and standard deviation, Limit of detection is calculated by using the following formula.[17]

$$\text{Limit of detection} = 10 \times \frac{\text{standard deviation of area value}}{\text{linearity curve slope value}}$$

- LOQ: ATO – 0.196 µg/ml; S/N level ratio – 10.6
- LOQ: MAF – 0.181 µg/ml; S/N level ratio - 3.6
- LOQ: ODE – 0.197 µg/ml; S/N level ratio – 10.1

3.1.4. Precision

ATO (16.67 g/ml), MAF (16.67 g/ml), and ODE (16.67 g/ml) solution concentrations were injected six replicates. Chromatograms were plotted by using optimized chromatographic conditions. The standard deviation, relative standard deviation for peak responses were calculated.

Table 3: Precision data.

ATO		MAF		ODE	
Area	**Data**	**Area**	**Data**	**Area**	**Data**
1801450	**Mean**	2795385	**Mean**	4041713	**Mean**
1805448	1809572	2793510	2799158	4047817	4038800
1817891	**SD**	2794882	**SD**	4037978	**SD**
1814119	6022.899	2805079	5328.907	4031317	6031.993
1807220	**RSD**	2800566	**RSD**	4033187	**RSD**
1811301	0.333	2805524	0.190	4040789	0.149

3.1.5. Accuracy

ATO (16.67 g/ml), MAF (16.67 g/ml), and ODE (16.67 g/ml) solution concentrations were injected six repeats in succession. The criterion specified in the segment "SETTINGS FOR ATO, MAF, & ODE COLLECTIVE ASSESSMENT" was used to assess. The peak response, assay and chromatograms related to ATO, MAF, & ODE were computed. The results of the tests were quite accurate for ATO, MAF, & ODE combinational analysis.

Table 4: Accuracy data.

Analyzed (µg/ml)	Determined (µg/ml)	Assayed (%)	Mean assay (%)
ATO			
16.67	16.45	98.67	99.12
16.67	16.48	98.89	
16.67	16.60	99.57	
16.67	16.56	99.37	
16.67	16.50	98.99	
16.67	16.54	99.21	
MAF			
16.67	16.48	98.88	99.01
16.67	16.47	98.81	
16.67	16.48	98.86	
16.67	16.54	99.22	
16.67	16.51	99.06	
16.67	16.54	99.24	
ODE			
16.67	16.57	99.39	99.32
16.67	16.59	99.54	
16.67	16.55	99.30	
16.67	16.52	99.13	
16.67	16.53	99.18	
16.67	16.56	99.36	

3.1.6. Recovery

ATO, MAF & ODE recoveries investigation included adding quantities of ATO, MAF & ODE standards to working Inmazeb™ solution with ATO quantity of 16.67 µg/ml, MAF quantity of 16.67 µg/ml and ODE quantity of 16.67 µg/ml.

Added quantities

1. 50% recovery stage: 8.252 µg/ml each of ATO, MAF & ODE
2. 100% recovery stage: 16.503 µg/ml each of ATO, MAF & ODE
3. 150% recovery stage: 24.755 µg/ml each of ATO, MAF & ODE

The samples of Inmazeb™ solution were tested three times by criterion specified in the segment "Settings for ATO, MAF, & ODE collective assessment", and the added ATO, MAF, and ODE amounts were computed by using relevant ATO, MAF, and ODE calibration curves. The peak response, recoveries and chromatograms related to ATO, MAF, & ODE were computed. The results of the tests were quite selective for ATO, MAF, & ODE combinational analysis.

Table 5: ATO recoveries.

Area	Add in (µg/ml)	Ascertained (µg/ml)	Assessed (%)	Mean assessed (%)
	50% recovery stage			
909729	8.252	8.31	100.66	100.68
909445	8.252	8.30	100.63	
910449	8.252	8.31	100.74	
	100% recovery stage			
1819845	16.503	16.62	100.69	100.01
1802329	16.503	16.46	99.72	
1800777	16.503	16.44	99.63	
	150% recovery stage			
2711780	24.755	24.76	100.02	100.23
2711261	24.755	24.76	100.00	
2729253	24.755	24.92	100.67	

Table 6: MAF recoveries.

Area	Add in (µg/ml)	Ascertained (µg/ml)	Assessed (%)	Mean assessed (%)
	50% recovery stage			
1388940	8.252	8.19	99.25	99.35
1395272	8.252	8.23	99.70	
1386625	8.252	8.18	99.08	
	100% recovery stage			
2805472	16.503	16.54	100.24	99.96
2797402	16.503	16.49	99.95	
2790595	16.503	16.45	99.70	
	150% recovery stage			
4207723	24.755	24.81	100.22	100.26
4213566	24.755	24.84	100.36	
4205817	24.755	24.80	100.18	

Table 7: ODE recoveries.

Area	Add in (µg/ml)	Ascertained (µg/ml)	Assessed (%)	Mean assessed (%)
	50% recovery stage			
2001828	8.252	8.21	99.45	99.79
2017925	8.252	8.27	100.25	
2006241	8.252	8.22	99.67	
	100% recovery stage			
4039731	16.503	16.56	100.34	100.30
4040310	16.503	16.56	100.36	
4034285	16.503	16.54	100.21	
	150% recovery stage			
6065089	24.755	24.86	100.43	

6060060	24.755	24.84	100.35	100.46
6075930	24.755	24.91	100.61	

3.1.7. Robustness

In effort to identify the degree of the robustness, the more significant chromatographic settings were tweaked, and the chromatographic equipment suitability profile was monitored and logged in parallel. The considered significant chromatographic settings were proportion of acetonitrile value of pH, detector nanometers, rate flow stream, set column temperature.Robustness was tried with ATO, MAF & ODE standard solution with ATO quantity of 16.67 µg/ml, MAF quantity of 16.67 µg/ml and ODE quantity of 16.67 µg/ml. The peak response, chromatographic equipment suitability profile and chromatograms related to ATO, MAF, & ODE were computed. The results of the tests were quite robust for ATO, MAF, & ODE combinational analysis.

3.1.8. Specificity

Inmazeb^{TM} solution with ATO quantity of 16.67 µg/ml, MAF quantity of 16.67 µg/ml and ODE quantity of 16.67 µg/ml, diluent [NaH_2PO_4, strength of 0.1M & pH 3.5 and acetonitrile (pure) are merged in a 40:60 vol/vol fraction] and Working ATO, MAF & ODE solution with ATO quantity of 16.67 µg/ml, MAF quantity of 16.67 µg/ml and ODE quantity of 16.67 µg/ml were analysed by criterisa. The peak chromatograms related to ATO, MAF, & ODE were computed. Excipient peaks and diluent related peaks were not detected among any of the LDE, DRE, or TFE retention periods. The results of the tests were quite selective for ATO, MAF, & ODE combinational analysis.

4. Degradation studies

The stock Inmazeb^{TM} solution with ATO quantity of 166.70 µg/ml, MAF quantity of 166.70 µg/ml and ODE quantity of 166.70 µg/ml was stressed consistent with ICH directives.

Table 8: Degradation data.

Condition	Area	Drug remained	Drug degraded
ATO			
Peroxide	1665970	91.25	8.75
Acid	1657614	90.79	9.21
Sun light	1699231	93.07	6.93
Alkali	1683216	92.20	7.8
Thermal	1637250	89.68	10.32
MAF			
Peroxide	2640371	93.39	6.61
Acid	2533352	89.61	10.39
Sun light	2677798	94.72	5.28
Alkali	2576889	91.15	8.85
Thermal	2561804	90.61	9.39
ODE			
Peroxide	3859963	94.92	5.08
Acid	3652703	89.82	10.18
Alkali	3750611	92.23	7.77
Sun light	3799085	93.42	6.58
Thermal	3683158	90.57	9.43

ATO stability

105 ^{o}C > HCl (0.1 N) > H_2O_2 (30%) > NaOH (0.1 N) > light.

MAF stability

HCl (0.1 N) > 105 ^{o}C > NaOH (0.1 N) > H_2O_2 (30%) > light.

ODE stability

HCl (0.1 N) > 105 ^{o}C > NaOH (0.1 N) > light > H_2O_2 (30%).

Stress breakdown compounds were not detected among any of the LDE, DRE, or TFE retention periods. The results of the tests were quite specific and stability indicating for ATO, MAF, & ODE combinational analysis.

5. CONCLUSION

This method can be used for simultaneous estimation of drug components of Inmazeb injection in quality control department for routine analysis. The combinational technique for Inmazeb injection was found having efficient, linear, reliable, robust and cost effective. There is a no interferences of additives or degradation products in quantification of drug components of the said formulation.

6. Conflict of interest

The authors report no conflicts of interest in this work.

7. ACKNOWLEDGMENT

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