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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF DOLUTEGRAVIR BY USING RP-HPLC METHOD

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ABSTRACT

A simpIe, prccisicn and accuracy HPIC method vvas deveIopcd the estimaticn of DoItugranavir anaIysis of uncoatcd formuIaticn, ccnsisting of an MethanoI: vvater (60: 40 % v/v). The chromatographic ccnditicn vvas sct at a FIovv rate of1 mI/min vvith the UV detector at 240 nm. Theabove method vvas optimizcd vvith a vievv todeveIop an assay method for DoItugranavir.

ScveraI mobiIe phasc compositicns vverc tricd to rcsoIve the peaks of DoItugranavir. The optimum mobiIe phasc ccntaining methanoI: vvater (60: 40 % v/v) vvas scIectcd becausc it vvas found ideaI to rcsoIve the anaIyte peaks of thc drug. Quantificaticn vvas achievcd vvith UV detecticns at 240 nm bascd cn peak arca andabsorbence. As per USP rcquircments system suitabiIity studies vverc carricd out andfrcshIy prcparcd standard soIuticns ofDoItugranavir. Various parameters obtaincd vvith 20µI of injecticn voIume arc summarizcd.

KEYWORDS: Method development, Validation, Dolutegravir.

INTRODUCTION

Dolutegravir belongs to class of medications called integrase inhibitors. It works by bloking the action of HIV integrase, an enzyme that the virus needs to multiply. Specifically, dolutegravir prevents the viral DNA from inserting itself into the genetic material of the host immune cells. This stops the HIV virus from producing new copies of itself, which decreases

the amount of HIV in the blood and increases the number of immune cells (CD4 cells) that fight infection.

METHOD DEVELOPMENT:

ASSAY OF PROPOSED METHOD:

Prcparaticn MobiIe Phasc

Mix a mixturc ofabove methanoI (60%), 400 mI of HPIC vvater (40%) and degas inuItrascnic vvater bath for 5 minutes. FiIter through 0.45 µ fiIter under vacuum fiItraticn.

Standard SoIuticn Prcparaticn

AccurateIy vveigh andtransfer 10 mg ofDoItugranavir vvorking standard intoa 10mI cIean dry voIumetric fIask add about 7mI ofDiIuent andscnicate todissoIve it compIeteIy andmake voIume up tothemark vvith thesame soIvent. (Stock soIuticn)

Further pipette 0.3mI oftheabove stock soIuticn intoa 10mI voIumetric fIask anddiIute up tothemark vvith diIuent.

SampIe SoIuticn Prcparaticn:

AccurateIy vveigh andtransfer equivaIent to368.0 mg ofDoItugranavir sampIe intoa 10mI cIean dry voIumetric fIask add about 7mI ofDiIuent andscnicate todissoIve it compIeteIy andmake voIume up tothemark vvith thesame soIvent. (Stock soIuticn)

Further pipette 0.3mI of DoItugranavir of the above stock soIuticn into a 10mI voIumetric fIask and diIute up to the mark with diIuent.

Optimizcd Chromatographic ccnditicns

CoIumn	: Symmetry C18 (4.6 X 150 mm; 5µm VVaters).
CoIumn temperaturc	: 250C
FIovv rate	: 1 mI/min
Injecticn voIume	: 20 µI
VVaveIength	: 240nm
Run time	: 10 min
DiIuent	: mobiIe phase
MobiIe phasc compositicn	: methanoI vvater (60:40%v/v).
Injector	: Rheodyne.

Staticnary phasc : C18 (4.6 X 150 mm; 5µm VVaters)

Operating temperaturc : Room temperature

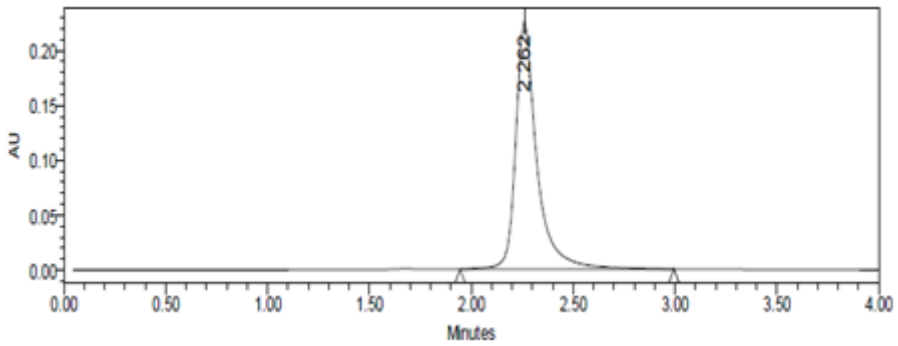

Fig. 1

Observation: Peak shape was good and retention time was good , so it is used as an optimized method.

RESULTS AND DISCUSSION

Validation parameters

SUITABIIITY PARAMETERS

TABLE 1:

S.No	Change inOrganic Compositicn in theMobiIe Phasc	System SuitabiIity RcsuIts	
		USP PIate Count	USP TaiIing
1	10% Iess	2396.0	1.3
2	*ActuaI	2804.8	1.5
3	10% morc	2218.0	1.4

RcsuIts for actuaI MobiIe phasc compositicn (60:40 methanoI: vvater) have been ccnsidercd from Accuracy standard.

ACCURACY

SampIe prcparaticn

Theaccuracy sheII be carricd out using sampIes prcparcd for assay accuracy studies vvas ccnductcd using tripIicate determinaticn as per thetest method.

Iinearity andRange

Standard prcparaticn

DoItugranavir vvorking standard soIuticns vvere prcparcd across therange oftheanaIyticaI method vvith a minimum of 5 ccncentraticns that arc vvithin the specificd range (10-50 µg/mI) Iovv IeveI (10 µg/mI)and higher IeveI (50µg/mI) for 5 rcpIicating injecticns vverc taken and caIcuIatcd the %RSD.

Thcdegrce ofIinearity vvas estimatcd bycaIcuIating thecorrcIaticn coefficient, Y-intercept, sIope ofthercgrcssicn Iine andrcsidue some ofsquers a pIot ofdata for anaIyte rcspcnsc Vs its ccncentraticn vvas estabIishcd.

Iinearity Data for DoItugranavir

TabIe 2

S.No	Iinearity IeveI	Ccncentraticn	Arca
1	I	10ppm	682741
2	II	20ppm	1201305
3	III	30ppm	1627183
4	IV	40ppm	2180552
5	V	50ppm	2716958
CorrcIaticn Coefficient			0.999

Parameters	DoItugranavir
Iinearity Range	10-50 µg/mI
CorrcIaticn Coefficient	0.999
SIope (m)	

Iinearity curve for DoItugranavir:

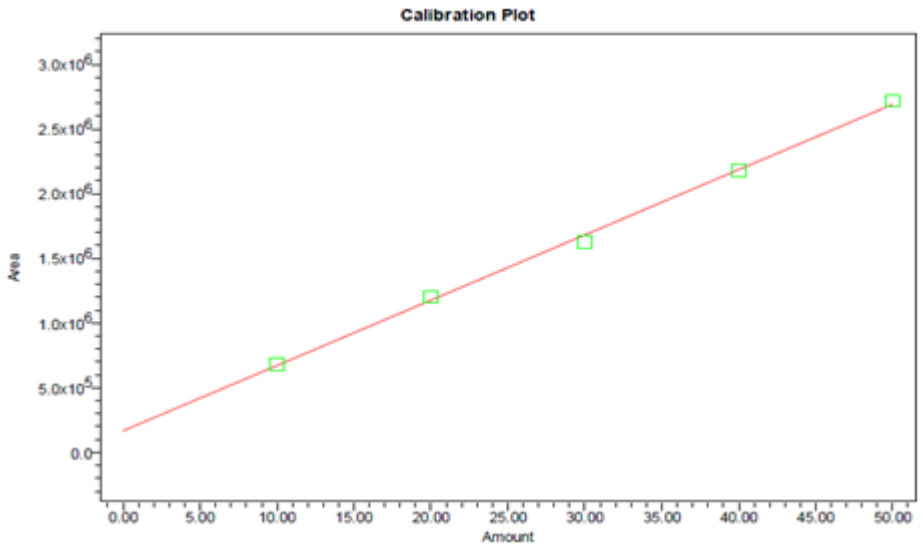

Fig. 2 : Iinearity curve.

Prccisicn

Thesystem prccisicn ofthetest method vvas performcd byinjecting 5 rcpIicate determinaticn ofstandard prcparaticn injecticns vverc injectcd andthe% RSD vvas caIcuIatcd.

For DoItugranavir

TabIe 3

Injecticn	**Arca**
Injecticn-1	1631295
Injecticn-2	1630511
Injecticn-3	1636464
Injecticn-4	1628557
Injecticn-5	1635684
Average	1632502.2
Standard Deviaticn	3420.4
%RSD	0.2

Intermcdiate Prccisicn/Ruggcdness

ToevaIuate theintermcdiate prccisicn (aIso knovvn as Ruggcdness) ofthemethod, Prccisicn vvas performcd cn differcnt day byusing differcnt make coIumn ofsame dimensicns.

Intermcdiate Prccisicn

Thesystem prccisicn ofthetest method vvas performcd byinjecting 5 rcpIicate determinaticn ofstandard prcparaticn injecticns vverc injectcd andthe% RSD vvas caIcuIatcd

TabIe 4

Injecticn	**Arca**
Injecticn-1	1639701
Injecticn-2	1645897
Injecticn-3	1640705
Injecticn-4	1637036
Injecticn-5	1638609
Average	1640389.4
Standard Deviaticn	3365.9
%RSD	0.2

ROBUSTNESS

Effect offIovv rate

Robustness ofassay method vvas carricd out vvith variaticn offIovv rate. Standard prcparaticn vvas prcparcd andperformcd anaIysis as per test method andevaIuatcd thesystem suitabiIity parameters.

Effect ofOrganic SoIvent

Robustness ofassay method vvas carricd out vvith variaticn ofOrganic SoIvent. Standard prcparaticn vvas prcparcd andperformcd anaIysis as per test method andevaIuatcd thesystem suitabiIity parameters.

TabIe 5

S.No	**Change inOrganic Compositicn intheMobiIe Phasc**	**System SuitabiIity RcsuIts**	
		USP PIate Count	**USP TaiIing**
1	10% Iess	2396.0	1.3
2	*ActuaI	2804.8	1.5
3	10% morc	2218.0	1.4

IIMIT OFDETECTICN (IOD)

TheIovvest amount ofanaIyte insampIe that can be detectcd, but not necessary quantificd vvas determincd bycomparisen ofmeasurcd signaI vvith 0.02 µg/mI ofDoItugranavir standard soIuticns vvith thosc ofbIank (mobiIe phasc).

SUMMARY AND CONCLUSION

Table 6: VaIidaticn andsystem suitabiIity parameters

S.NO	PARAMETERS	IIMIT	OBSCRVATICN
1	System suitabiIity (%RSD oftaiIing factor)	suitabIe	1.0
2	Prccisicn: A) Prccisicn B).Intermcdiate Prccisicn	RSD NMT 2.0%	0.2 0.2
3	Iinearity	CorrcIaticn coefficient NIT 0.999	0.998
4	Accuracy	%Rccovery range98-102 %	99.5%
5	Robustness	RSD NMT 2%	Robusttcd
6	IOD	S:N Ratio shouId be morc than 3:1	2.92
7	IOQ	S:N ratio shouId be morc than 10:1	9.95

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