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Simultaneous Estimation of Azacitidine- Viltolarsen Bulk Drugs and Dosage Form by HPLC and Spectrophotometric Techniques

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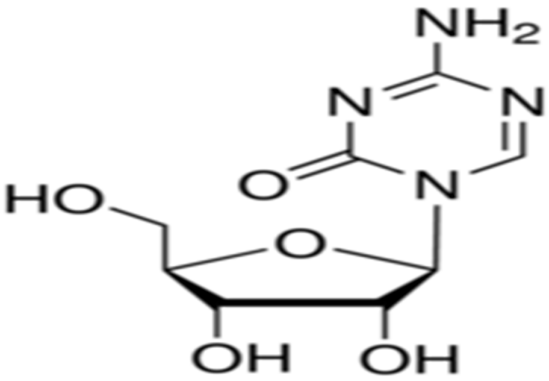
Abstract: *The present communication deals with the development of a new drug, simple, specific, sensitive, rapid and economical procedure for simultaneous estimation of Azacitidine and Viltolarsen in a combined dosage form. The method is based on the native ultraviolet absorbance maxima of the two chemotherapeutic agents. As both compounds do not interact chemically in methanol, two wavelengths 240 nm for Azacitidine and 240 nm for viltolarsen were used. Both the drugs obeyed Beer's law and LOQ and LOD in the concentration range that was employed in the method.*

Keywords: Azacitidine, Viltolarsen HPLC and Spectrophotometric

I. INTRODUCTION

1.1 Azacitidine



Structures of the Azacitidine:

A. Common Side Effect and Medicinal Purpose Uses

The two drugs were first evolved in Czechoslovakia as an expected chemotherapy for malignancy. Azacitidine is the primary drug used to treat myelodysplastic disorder (MDS) since it has been affirmed by the US Food and Drug Administration. It is showcased under the name Vidaza. In a randomized controlled preliminary contrasting azacitidine and MDS steady treatment, approx. 16% of drug beneficiaries had a fractional or complete reaction to platelets and bone marrow morphology got back to business as usual and 2/3 of patients who required a blood bonding for an examination were not, at this point required subsequent to accepting azacitidine. It can likewise be checked in vitro to eliminate methyl bunches from DNA. This can diminish the effect of the development of measures to decrease methylation. Thusly, DNA methylation occasions are accepted to guarantee stagnation. Disinfecting can diminish the dependability of sedation signs and in this way have a general hereditary force. Portrayed the sensational impact of 5-azacitidine on freak IDH1 gliomaxenography in mice.

A few logical techniques have been unveiled for the assurance of azacitidine in organic liquids, including great fluid chromatography and fluid chromatography spectroscopy. Until this point in time, no strategy has been distributed for the assurance of azacitidine in a drug piece for testing for pharmacokinetic or pollutants.

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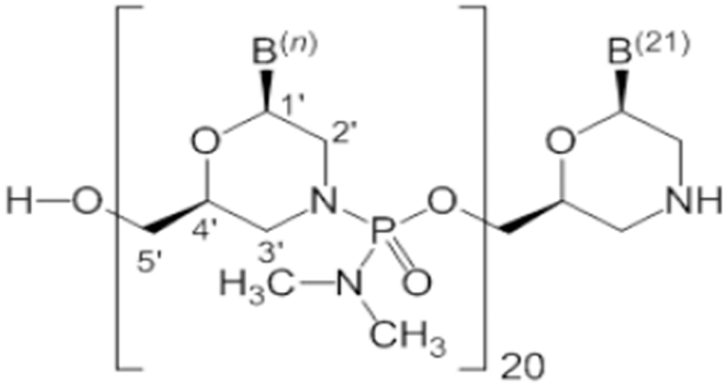
1.2 Viltolarsen



Structures of the viltolarsen

A. Common Side Effect & Medicinal Purpose Uses:

Viltepso (viltolarsen) with antisense oligonucleotides is a fine for the treatment of Dyschen muscular dystrophy (DMD) in patients with confirmed mutations in the DMD gene, which is suitable for exon 53 for bypass. The molecules of DNA synthetic KP are to be found naturally in RNA and RNA cat ribofuranosyylirene replace it rings a mhoirfínín members. However, the phosphorodiamidate residue contains morphine due to the presence of negative phosphate compounds in DNA and RNA. Each morpholinophosphorodiamidate subunit contains one of the heterocyclic bases present in DNA (adenine , cytosine , guanine or Tim). VILTEPSO indication for treatment of patients with Duchenne muscular dystrophy (DMD).

II. Structural Formula of Viltolarsen

VILTEPSo is injected without preservation into sterile topographic water for intravenous use. VILTEPSO is a clear and colorless solution. VILTEPSO is administered in a dose of 250 mg / 5 ml of tar filter (50 mg / ml) 0.9 % sodium chloride. Each ml of VILTEPSO contains 50 mg of vildolar sodium chloride and 9 mg of water for injections. The final pH of the product was 7.0 to 7.5 times the chloroform content in sodium hydroxide. Anti sense volters and oligonucleotides in the subclass of morpholinophosphorodiamide (PMO) polymer. The molecules of DNA synthetic KP are to be found naturally in RNA and RNA cat ribofuranosyylirene replace it rings a mhoirfínín members. However, the phosphorodiamidate residue contains morphine due to the presence of negative phosphate compounds in DNA and RNA. Each morpholinophosphorodiamidate subunit contains one of the heterocyclic base present in DNA (adenine , cytosine , guanine or Tim). 21 devices are connected in game victims. Viltolarsen takes the molecular formula C 244 H 381 N 113 O 88 P 20 with a molecular weight of 6924, 82 daltons. VILTEPSO indication for treatment of patients with Duchenne muscular dystrophy (DMD) Code P DMD mutation confirmation for right bypass exon 53. This provides insight into accelerated approval based on production binding in skeletal muscular dystrophy observed in patients treated with VILTEPSO. Further approval of medicinal product obtained in confirmatory studies. Dose data The initial dose of VILTEPSO is 80 mg / kg daily and an intravenous infusion of 60 minutes. When a dose of VILTEPSO is administered, these data are presented in the next dose.

2.1 Objectives of the Work

1. To develop method for azacitidine and viltolarsen.
2. To validate the developed method of viltolarsen and azacitidine.

III. Instruments and Chemicals

The instruments and chemicals which were required to perform this study are as shown in table:

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List of Instrument

SR. NO.	TOOL	MANUFACTURER
1.	HPLC	Shimadzu (Prominence-I LC-2030C 3D)
2.	UV	Shimadzu (UV- Pharmaspec 1700)
3.	pH Meter	Lab india
4.	Analytical Balance	Sartoriuscubis
5.	Milli-Q Water	Milli-Q (Millipore)

List of Device

SR. NO.	APPARATUS	MANUFACTURER
1.	Volumetric flask	Rankem
2.	Beaker	Borosilicate Glass
3.	Pippete	Borosilicate Glass
4.	Measuring cylinder	Tarson

List of Reagents

SR.NO	REAGENT	MANUFACTURER
1	Acetonitrile	Merck life science, Mumbai
2	Triflouro acetic acid	Merck life science, Mumbai

IV. RESEARCH METHODOLOGY

In this chapter various techniques and methods is described which are used to perform the needed experiment and analysis of the work. Starting from the The Gas chromatography and then followed by various techniques and the experiment performed. Gas chromatography, High performance liquid chromatography, paper chromatography, thin layer chromatography etc.

- Arrangement of standard arrangements of azacitidine: A stock arrangement of azacitidine (0.8 mg•mL–1) was set up by dissolving a suitable sum in diluent. A stock arrangement of pollutions (0.8 mg•mL–1) at a centralization of (0.8 mg•mL–1) was additionally set up in diluent. Working arrangements were set up from above stock answer for related substances assurance and test assurance, separately.
- Readiness of test answer for azacitidine: The jar was set on turning shaker for 10 min and sonicated for 10 min to disintegrate the material totally. The supernatant arrangement was gathered and sifted.
- Chromatographic conditions for azacitidine: The chromatographic condition follows an angle program comprising of 3.1g of Ammonium acetic acid derivation in 1000 mL of water and blended well. The pH of the arrangement was acclimated to 6.4±0.05 with weaken acidic corrosive and blended well and utilized as portable stage A. Combination of versatile The inclination program was: Time/% versatile stage B is 0.0/0, 15/0, 30/20, 45/40, 55/50, 60/0. The stream pace of the portable stage is 1.0 mL min-1.
- Particularity: Explicitness is the capacity of the technique to quantify the analyte reaction within the sight of its likely pollutions. Stress testing of the drug substance can assist with recognizing the presumable debasement items, which can thusly assist with setting up the corruption pathways and characteristic dependability of the atom and approved the security showing force of the insightful systems utilized.

The explicitness of azacitidine within the sight of its debasements specifically demon A, pixie B, devil C, devil D and corruption items was controlled by the created HPLC strategy.

4.1 Equipment Used

Waters alliance equipped HPLC system with a photo diode array detector is used for the method development and force degradation studies. The HPLC system used for method validation is waters HPLC system with variable wavelength detector (VWD) and Shimadzu 2010 series LC system with UV detector. The data is monitored and processed by using LC-solution Software. The chromatographic column used is YMC pack ODS-AQ, (250 mm x 4.6 mm 5µm).

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V. Results and Discussion

Chromatographic condition for Azacitidine-Viltolarsen:

Method Development and Optimization

The new HPLC technique is advanced so as to build up a steadiness showing strategy for azacitidine and its pollutions. 3.1g of ammonium acetic acid derivation in 1000 mL of water and blended well. The pH of the arrangement is acclimated to 6.4±0.05 with weaken acidic corrosive and blended well and utilized as portable stage A.

In this path was fewer goals between pollutant C and debasement D and the two contaminations maintenance times are exceptionally high. In another path changes were made on versatile stage B proportion (50:25:25) and infused test is then spiked and the inclination was changed to diminish longer run season of azacitidine and different debasements. With this path less maintenance season of azacitidine was accomplished and top states of contaminations were additionally improved. In view of second path another path was completed by utilizing YMC ODS AQ-5, (250 x 4.6 mm), 5 µm molecule size, with section temperature 35°C. The goal among pollutions and azacitidone is <2.0. To additionally improve the goal among debasements and azacitidine, again we changed the versatile stage B (50:30:20) proportion. The all pollutions were very much settled the goal somewhere in the range of 2.0 and 3.0. Acceptable goal among contaminations and evenness of the azacitidine top was seen in finaltrail.

5.1 LOD and LOQ

The constraint of recognition and breaking point of measurement were assessed by successive weakening of amlodipine besylate and rosuvastatin in calcium answer for acquire a sign to-commotion proportion of RP-HPLC Chromatogram of Azacitidine and Viltolarsen in water and 0.1 % TFA and Acetonitrile and 0.1%TFA. (Gradient start with 70:30) Flow Rate: 1.0 ml/min at 240 n

Gradient Programme:

Time (min)	Movable phase A	Portable phase B
0.0	70	30
6.0	70	30
6.1	55	45
15.0	55	45
20.0	50	50
20.1	70	30
25.0	70	30

5.2 Scheme Correctness Stricture

Table: System Suitability Parameter for Azacitidine and Viltolarsen

Sr No	Constraint	Viltolarsen	Azacitidine
1	Retention time	6.42	14.75
2	Theoretical plates	13370	61010
3	Tailing factor	1.26	1.14
4	Resolution	20.17	

5.3 Linearity

Azacitidine			
Level	Conc. In µg/ml	Mean Areas±SDs (n is equal to 6)	%RSD
50%	81.1	2659799±2659.7	0.10
80%	129.8	4254483±6381.7	0.15
100%	162.3	5328473±10656.9	0.20
120%	194.7	6420188±5778.1	0.09
150%	243.4	7993045±14387.4	0.18
Correlation (R)	0.999		

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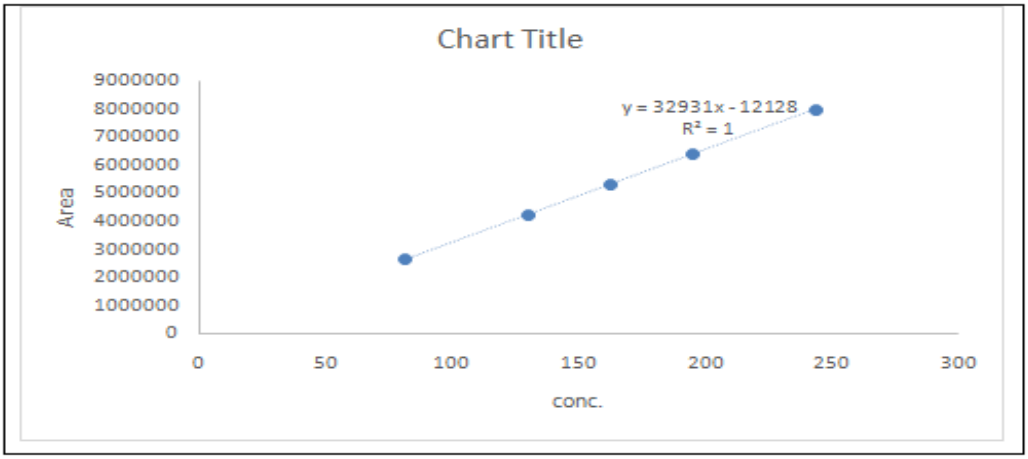

Figure: Calibrations Based curve of Azacitidine (81.1 – 243.4 µg/ml)

Table: Linearity of Viltolarsen

Viltolarsen			
Level	**Conc. In µg/ml**	**Mean Areas±SDs (n is equal to 6)**	**%RSD**
50%	**55.5**	**2343988±2812.7**	**0.12**
80%	**92.6**	**3938988±8665.7**	**0.22**
100%	**115.7**	**4879243±3903.3**	**0.08**
120%	**138.9**	**5839291±583.9**	**0.01**
150%	**185.1**	**7746147±7746.1**	**0.10**
Correlation (R)	**1**		

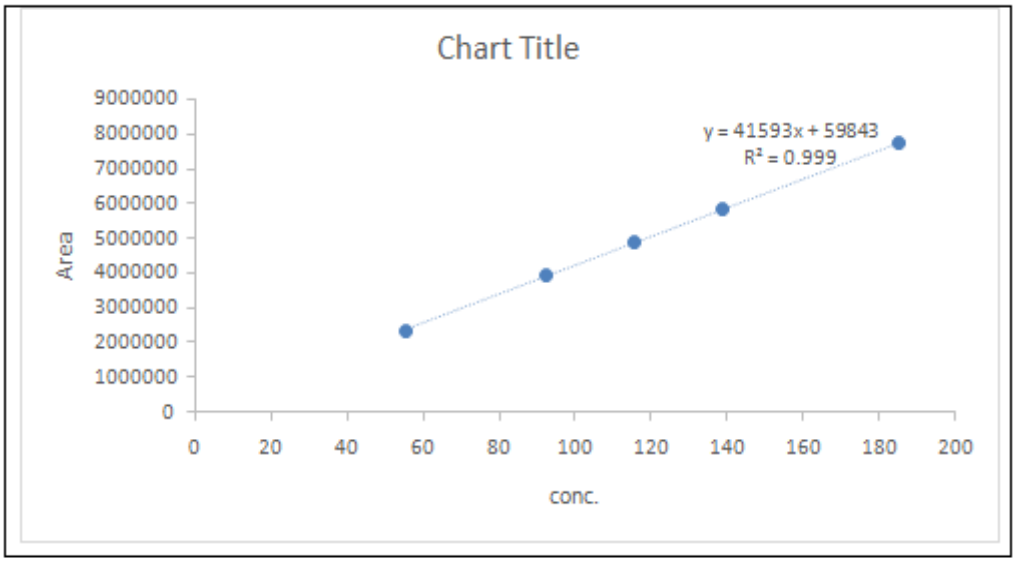

Figure: Calibrations Based curve of Viltolarsen (55.5 – 185.1 µg/ml)

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5.4 Accuracy

Table: Accuracy study data of Azacitidine and Viltolarsen

Drug	Level	Area	ml added	Amt. added (mg)	Amt. Recovered (mg)	% Recovery	Avrg. ±SD	% RSD
Azacitidine	50%	2698648	1.5	4.05	4.09	99.02	99.74± 0.6235	0.62
		2646135	1.5	4.05	4.01	100.1		
		2642525	1.5	4.05	4.01	100.1		
	100%	5311809	3.0	8.11	8.06	100.6	100.6± 0.1000	0.09
		5303907	3.0	8.11	8.05	100.7		
		5304729	3.0	8.11	8.07	100.5		
	150%	8075400	4.5	12.16	12.26	99.18	99.07±0.0923	0.09
		8083433	4.5	12.16	12.28	99.02		
		8083688	4.5	12.16	12.28	99.02		
Viltolarsen	50%	2351976	1.2	2.77	2.77	99.63	100.1±0.3868	0.3
		2344833	1.2	2.77	2.76	100.3		
		2344836	1.2	2.77	2.76	100.3		
	100%	4870403	2.5	5.79	5.74	100.8	101.2±0.3464	0.34
		4849744	2.5	5.79	5.71	101.4		
		4851364	2.5	5.79	5.71	101.4		
	150%	7829117	4.0	9.26	9.22	100.4	100.4±0.1000	0.09
		7838052	4.0	9.26	9.23	100.3		
		7825015	4.0	9.26	9.21	100.5		

5.5 Precision

Table: Assay Precision

Set	Area of Azacitidine	Assay of Azacitidine	Area of Viltolarsen	Assay of Viltolarsen
1	5462798.000	100.4 %	5096846.000	103.4%
2	5465304.000	100.5 %	5111770.000	103.7 %
3	5472345.500	100.6 %	5120940.500	103.8 %
4	5473372.000	100.6 %	5104933.000	103.5 %
5	5462360.000	100.4 %	5097442.000	104.5 %
6	5474470.000	100.6 %	5102382.000	103.5 %
Average	5468442	100.5 %	5105719	103.6 %
Standard Deviation	5560.063	0.0898	9244.467	0.1500
%RSD	0.10	0.10	0.18	0.10

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