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THE ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF POVIDONE & ORNIDAZOLE BY USING RP- HPLC

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Abstract:
A new method was established for simultaneous estimation of Povidone and Ornidazole by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Povidone and Ornidazole by using Inertsil C18 column (4.6×150mm)5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol: Phosphate buffer, detection wavelength was 240 nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The retention times were found to be 2.462 mins and 3.737 mins. The % purity of Povidone and Ornidazole was found to be 99.84% and 100.27% respectively. The system suitability parameters for Povidone and Ornidazole such as theoretical plates and tailing factor were found to be 5358.3, 1.2 and 7597 and 1.1, the resolution was found to be 8.4. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Povidone and Ornidazole was found in concentration range of 10µg-50µg and 25µg-125µg and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 99.96% and 99.98%, %RSD for repeatability was 0.3 and 0.7, % RSD for intermediate precision was 0.3 and 0.9 respectively. The precision study was precision, robustness and repeatabilty.LOD value was 3.17 and 0.2372 and LOQ value was 7.80 and 5.30 respectively.

Keywords: *Povidone , Ornidazole ,precision, robustness and repeatability.*

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INTRODUCTION:

Povidone is White coloured powder, which is having a **Molecular formula of** $C_6H_{13}NOP_2$ and**Molecularweight:**177.121 belongs to category of disintegrant which was soluble in water. The antimicrobial activity of povidone-iodine happens after iodine disassociates from the complex. Once in the free structure, iodine quickly enters microbial cell membrane and collaborates with proteins, nucleotides, and unsaturated fats in the cytoplasm and cytoplasmic membrane. **ORNIDAZOLE which was a** white colour crystalline powder and having the molecular formula of C7-H10-Cl-N219. which was highly soluble in water and which act as Antibacterial agent. The analysis of Povidone and Ornidazole by simultaneous estimation by RP-HPLC. Spectrophotometer, HPLC and HPTLC are the reported analytical methods for compounds either individually or in combination with other dosage form. Hence, it was felt that, there is a need of new analytical method development for the simultaneous estimation of Povidone and Ornidazole in pharmaceutical dosage form. Present work is aimed to develop a new, simple, fast, rapid, accurate, efficient and reproducible RP-HPLC method for the simultaneous analysis of Povidone and Ornidazole. The developed technique determination be validated conferring to ICH guidelines [1].

METHOD AND METHODOLOGY [3-7]:

List of chemicals and standards used

S.No	Chemicals	Manufacturer Name	Grade
1.	Water	Merck	HPLC grade
2.	Methanol	Merck	HPLC grade
3.	Acetonitrile	Merck	HPLC grade
4.	Ortho phosphoric acid	Merck	G.R
5.	KH_2PO_4	Merck	G.R
6.	K_2HPO_4	Merck	G.R
7.	0. 22µ Nylon filter	Advanced lab	HPLC grade
8.	0.45µ filter paper	Millipore	HPLC grade
9.	Povidone and Ornidazole	In – House	In- House

List of instruments used

S.No	Instrument name	Model number	Soft ware	Manufacturers Name
1	HPLC-auto sampler –UV detector	Separation module2695, UV.detector2487	Empower-software version-2	Waters
2	U.V double beam spectrometer	UV 3000+	U.V win soft ware	Lab India
3	Digital weighing balance (sensitivity 5mg)	ER 200A	-	Ascoset
4	pH meter	AD 102U	-	ADWA
5	Sonicator	SE60US	-	Enertech

Procedure

Preparation of phosphate buffer

2.95 grams of $KH_2PO_4$and 5.45 grams of K_2HPO_4 was weighed and taken into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water and pH was adjusted to 3 with orthophosphoric acid. The resulting solution was sonicated and filtered.

Preparation of mobile phase

Mix a mixture of above buffer 300 ml (30%) and 700 ml of methanol (HPLC grade-70%) and degassed in ultrasonic water bath for 5 minutes. Filter through 0.22 µ filter under vacuum filtration.

Diluents preparation

Mobile phase was used as the diluent.

Preparation of the individual Povidone standard preparation

10 mg of Povidone working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 0.2ml from the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent. Final concentration is 20µg/ml.

Preparation of the individual Ornidazole standard preparation

10mg of Ornidazole working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to

Dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette out 0.4ml from the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent. Final concentration is 40 µg/ml.

Preparation of the Povidone and Ornidazole standard and sample solution

Sample solution preparation:

An equivalent tablet power such that 10mg of Povidone and 25mg Ornidazole tablet powder were accurately weighed and transferred into a 10ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent(Stock solution). Further pipette 10ml of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

Standard solution preparation

10 mg Povidone and 25mg Ornidazole working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

Procedure

10µL of the blank, standard and sample were injected into the chromatographic system and areas for the Povidone and Ornidazole the peaks were used for calculating the % assay by using the formulae.

System suitability

- Tailing factor for the peaks due to Povidone and Ornidazole in standard solution should not be more than 1.5.
- Theoretical plates for the Povidone and Ornidazole peaks in standard solution should not be less than 2000.

Assay calculation

$$\textbf{Assay}\ \% = \frac{\textbf{samplearea}}{\textbf{Standardarea}} \times \frac{\textbf{dilutionsample}}{\textbf{dilutionofstandard}} \times \frac{\textbf{P}}{\textbf{100}} \times \frac{\textbf{Avg.wt}}{\textbf{Lc}} \times \textbf{100}$$

Where:

Avg.wt = average weight of tablets

P= Percentage purity of working standard

LC= Label Claim of Povidone mg/ml.

Chromatographic trials for simultaneous estimation of Povidone and Ornidazole by RP- HPLC

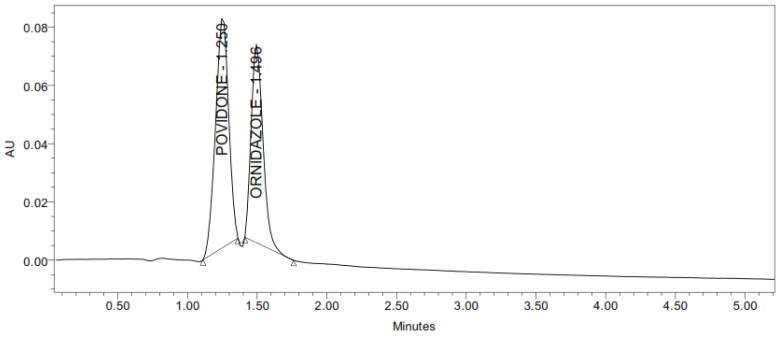
	Trail -1	Trail 2	Trail 3	Trail 4	Trail 5 **optimised method**
Mobile phase	ACN: H2O (70:30%v/v)	Methanol: H_2O (70:30%v/v)	ACN: Methanol (20:80 % v/v)	ACN: Methanol (30:70% v/v)	Methanol: Phosphate buffer pH4.0 (70: 30 % v/v)
Diluent	Mobile phase was used as the diluent	Mobile phase was used as the diluent	Mobile phase was used as the diluent	Mobile phase was used as the diluent	Mobile phase was used as the diluent
Flow rate	1ml/min	1.0ml per min	1.0 ml per min	1.0 ml per min	1.0 ml per min
Column	Symmetry C184.6x150mm, 5µm	Inertsil C18 4.6x150mm 5µm	Thermosil RPC8 4.5×150mm 5.0 µm	Agilent RPC18 4.6×250mm 5µm	InertsilC18 column (4.6×150mm) 5µ µm
Detector wavelength	240nm	226nm	225nm	225nm	240nm
Column oven	Ambient	Ambient	Ambient	Ambient	Ambient
Injection volume	10µl	20µl	10µl	20µl	10µl
Running time	5min	10 min	6 min	10min	10min

Validation parameters such as specificity, Linearity, Range, Accuracy, Precision, Repeatability, Intermediate Precision , Detection Limit, Quantitation Limit, Robustness also studied [5-10].

RESULTS AND DISCUSSION:

Chromatographic trials for simultaneous estimation of Povidone and Ornidazole by RP- HPLC.

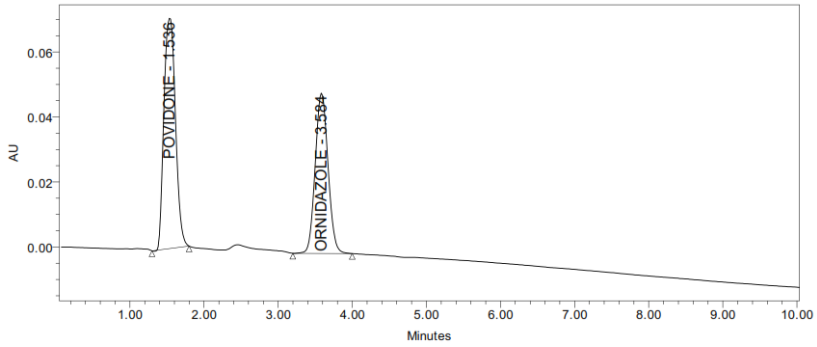
Trial-1: Chromatogram showing trial1 injection



Observation:

The trial shows no proper separation of peaks in the chromatogram, so more trials were required for obtaining peaks.

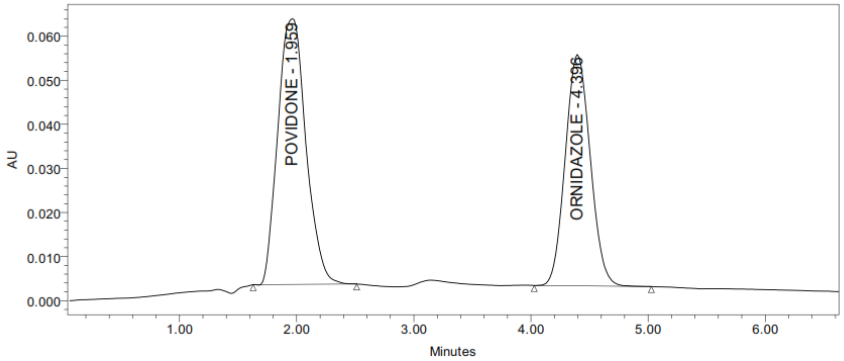
Chromatogram showing trial-2 injection



Observation

In this trial Both peaks were eluted well, But there was no proper baseline.Need some more trails.

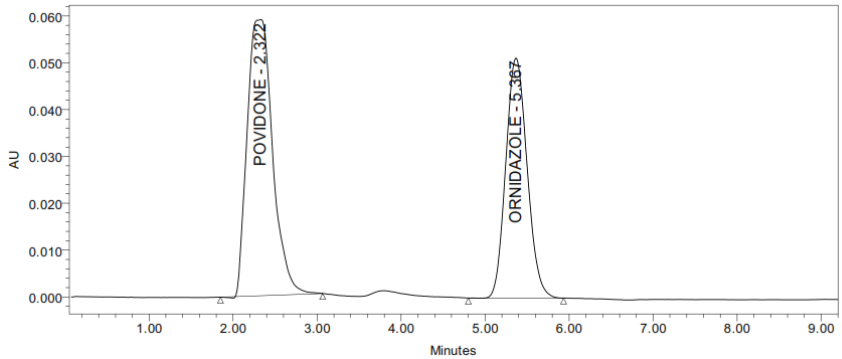
Trial-3 : Chromatogram showing trial-3 injection



Observation

In this trial both Povidone and Ornidazolewere eluted but there is no proper resolution. Still more trials were required for better resolution in peaks.

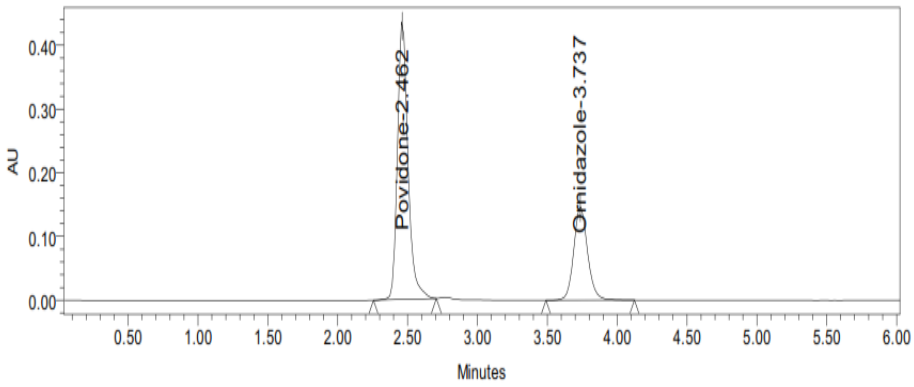
Trial-4:Chromatogram showing trial-4 injections



Observation:

The separation was good; peak shape was good, still more trials were required to reduce the retention times of peaks.

Trial -5 (optimised method): Chromatogram showing trial-5 injection



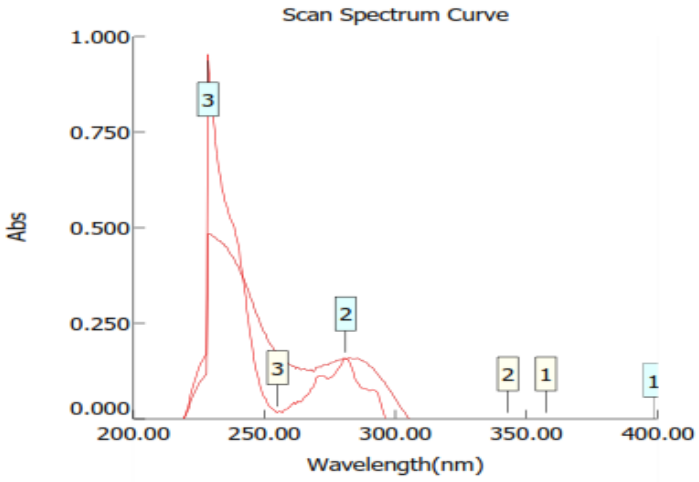
Observation

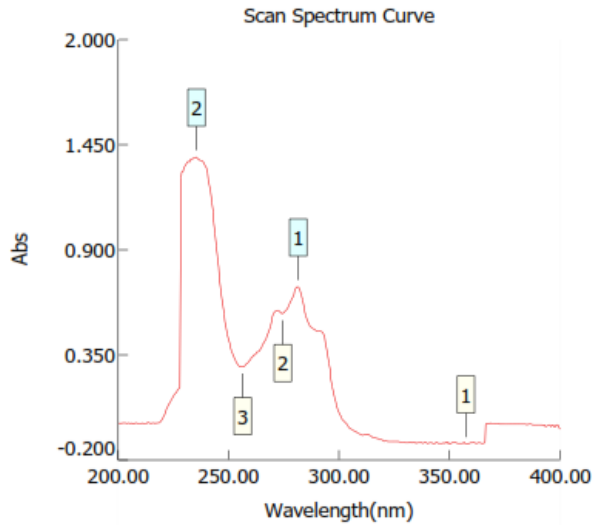
The separation was good, peak shape was good, so we conclude that there is no required for reduce the retention times of peaks,so it is taken as final method.

Method Development:

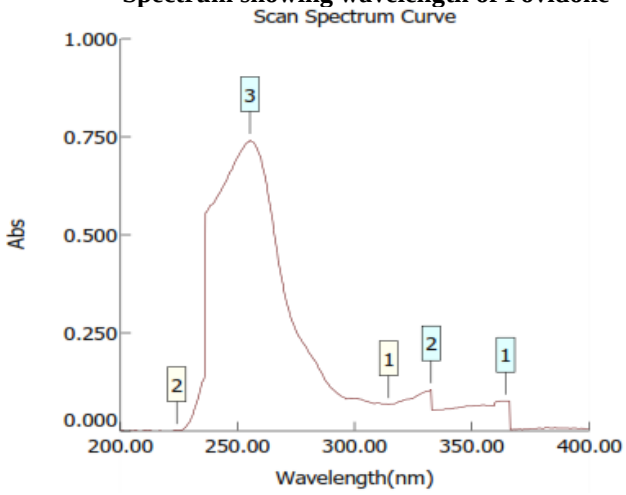
The identification wavelength were selected by dissolving the drug in mobile phase to get a concentproportionn of 10µg/ml for individual and mixed standards. The resulting solution were scanned in U.V range from 200-400nm. The overlay spectrum of Povidone and Ornidazolewere obtained and the isobestic point of Povidone and Ornidazoleshowed absorbance's maxima at 240nm. The spectrums are shown below

Spectrum showing overlapping spectrum of Povi and Orni





Spectrum showing wavelength of Povidone

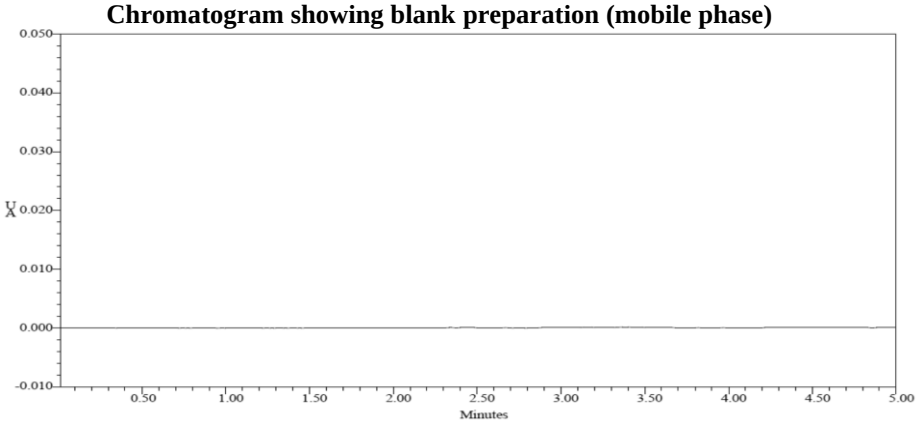


Spectrum showing wavelength of Ornidazole

The chromatographic method development for the simultaneous estimate of Povidone and Ornidazole were optimized by several trials for various parameters as different column, flow rate and mobile phase, finally the following chromatographic method were selected for the sepaproportionn and quantification of Povidone and Ornidazole in API and pharmaceutical dosage form by RP-HPLC method.

Optimized chromatographic conditions for simultaneous estimates of Povidone and Ornidazole by RP-HPLC method

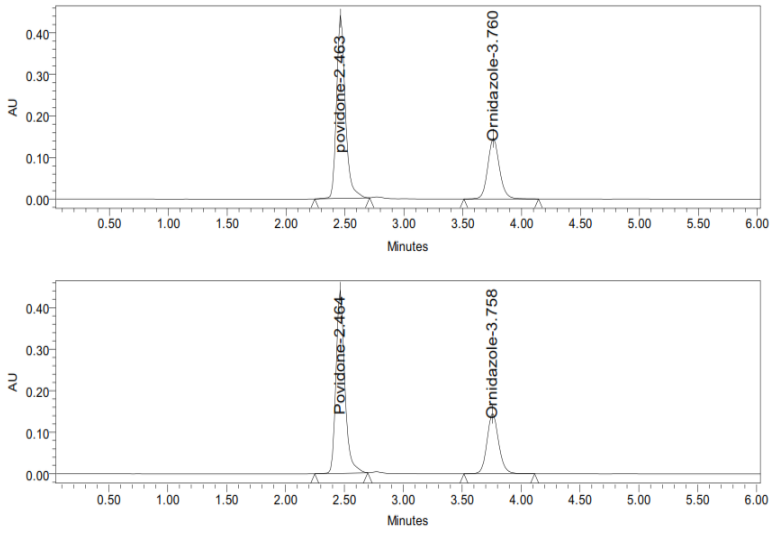
Chromatogram showing blank preparation (mobile phase)



Assay calculation for Povidone and Ornidazole

The assay study were performed for the Povidone and Ornidazole. Each three injections of sample and standard were injected into chromatographic system. The chromatograms are shown in below

Chromatogram showing assay of sample injection-1,2,3



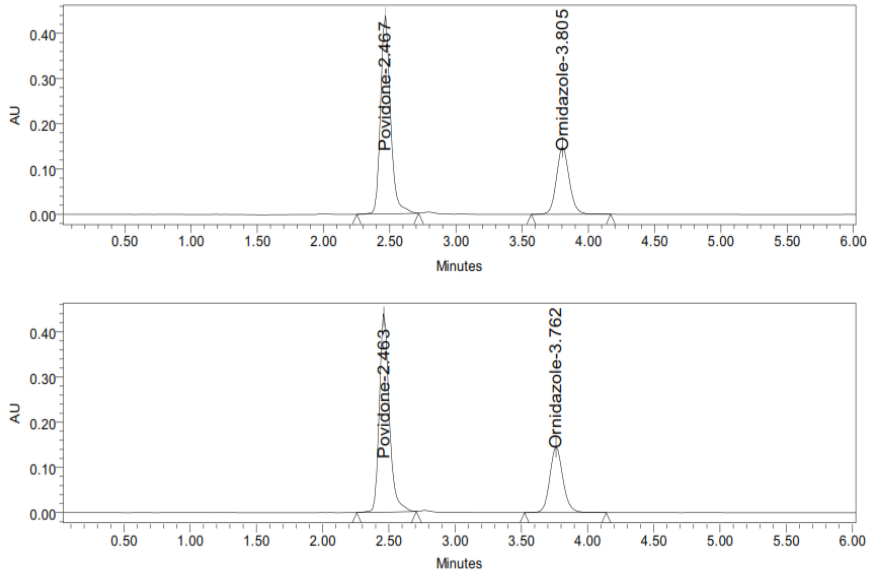
Peak Name: Povidone

	Peak Name	RT	Area (µV*sec)	Height (µV)
1	Povidone	2.464	2286043	434142
2	Povidone	2.463	2288873	435418
Mean			2287458.0	
Std. Dev.			2001.0	
% RSD			0.1	

Peak Name: Ornidazole

	Peak Name	RT	Area (µV*sec)	Height (µV)
1	Ornidazole	3.758	1003783	144483
2	Ornidazole	3.760	1006018	145772
Mean			1004900.6	
Std. Dev.			1580.6	
% RSD			0.2	

Chromatogram showingstandard of sample injection -1,2,3.



Peak Name: Povidone

	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Povidone	2.467	2285821	433152	5358.3	1.2
2	Povidone	2.463	2294216	435435	5450.9	1.3
Mean			2290018.5		5404.6	1.2
Std. Dev.			5935.5			
% RSD			0.3			

Peak Name: Ornidazole

	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing	USP Resolution
1	Ornidazole	3.805	1004856	148700	7597.5	1.1	8.4
2	Ornidazole	3.762	1006174	145751	7091.2	1.1	8.2
Mean			1005514.9		7344.3	1.1	
Std. Dev.			931.7				
% RSD			0.1				

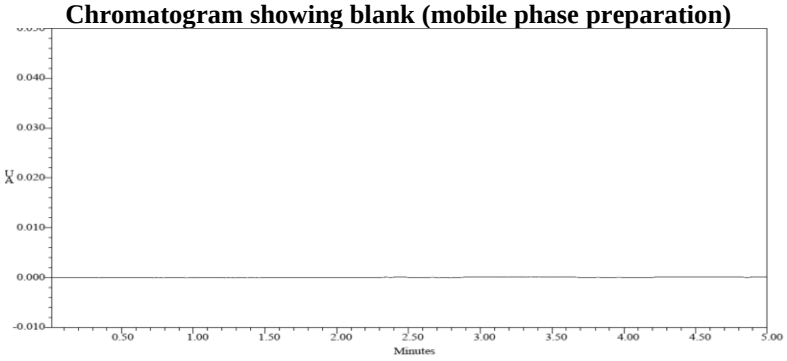
The retention time of Povidone and Ornidazolewere found to be 2.467 mins and 3.805mins individually.The system suitability parameters for Povidone and Ornidazolesuch as theoretical plates and tailing factor were found to be 5358.3, 1.2 and 7597.5,1.1.Resolution were8.4.The % purity Povidone and Ornidazolein pharmaceutical dosage form were found to be 99.84 and 100.27% individually.

VALIDATION REPORT:

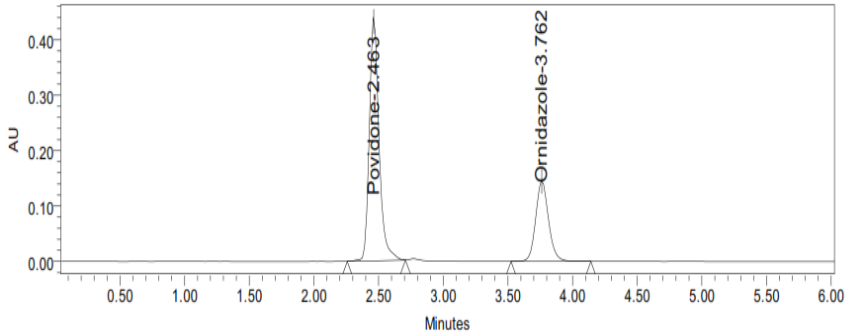
Specificity

The system suitability for specificity were carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The study were performed by injecting blank. The chromatograms are shown in Fig below

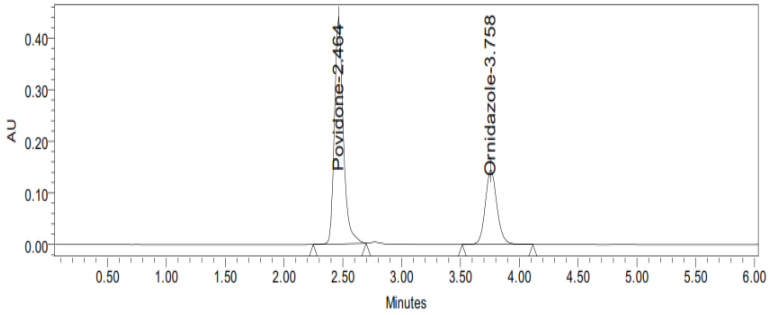
Chromatogram showing blank (mobile phase preparation)



Chromatogram showing standard injection



Chromatogram showing sample injection

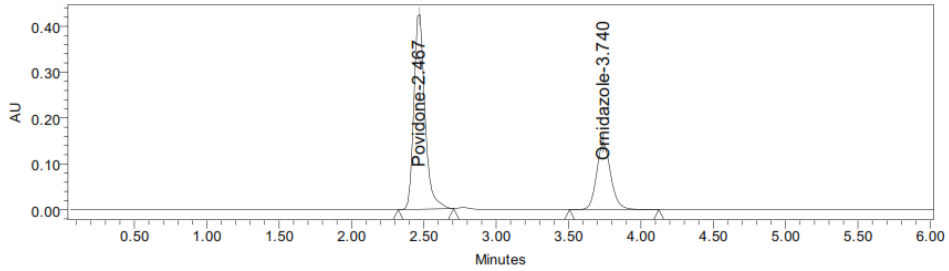


The specificity test were performed for Povidone and Ornidazole. It were found that there were no interference of impurities in retention time of analytical peak.

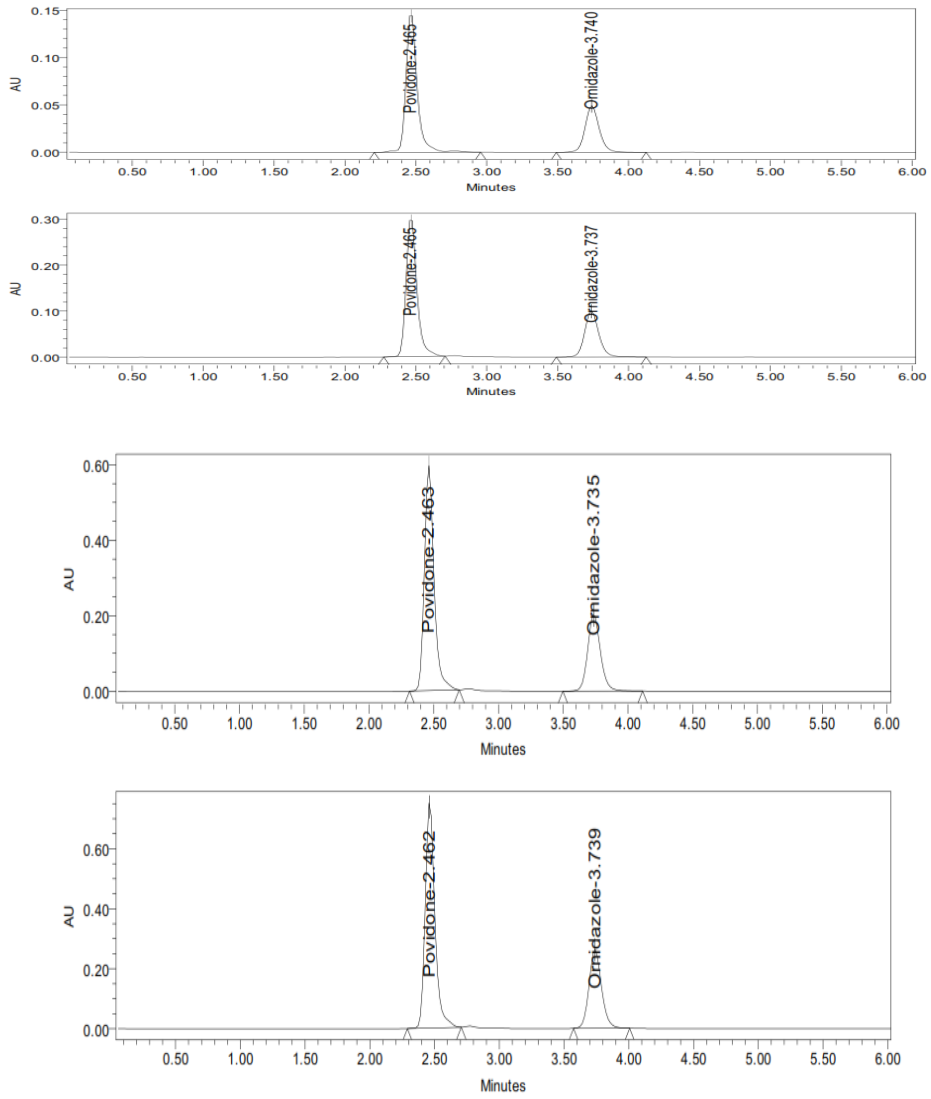
Linearity:

The linearity study were performed for the concentproportionn of 10 ppmto 50 ppmPovidone and 25 ppm to 125ppmOrnidazole level. Each level were injected into chromatographic system. The area of each level were used for calculation of correlation coefficient. The results are tabulated below Calibproportionn graph for EMPA and LINA are shown below

. Chromatograms showing linearity level-1 to level 5 (10ppm-50 ppm of Povidone and25ppm -125ppm of Ornidazole) injections.



SI.NO	Linearity level	Concentproportionn	Area
1	I	20 ppm	784928
2	II	40ppm	1524159
3	III	60ppm	2329360
4	IV	80ppm	3065982
5	V	100ppm	3830623
Correlation Coefficient			0.999

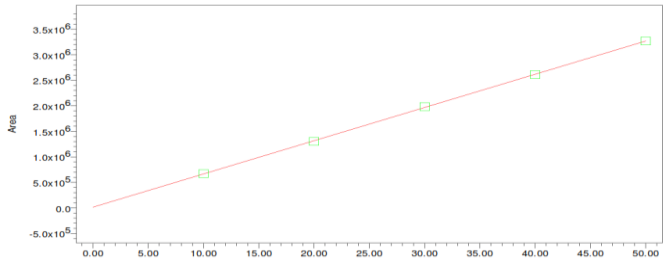


Linearity Results for Povidone:

S.No	Linearity Level	Concentproportionn	Area
1	I	10 ppm	668978
2	II	20 ppm	1303018
3	III	30 ppm	1984694
4	IV	400 ppm	2611374
5	V	50 ppm	3269630
Correlation Coefficient			0.999

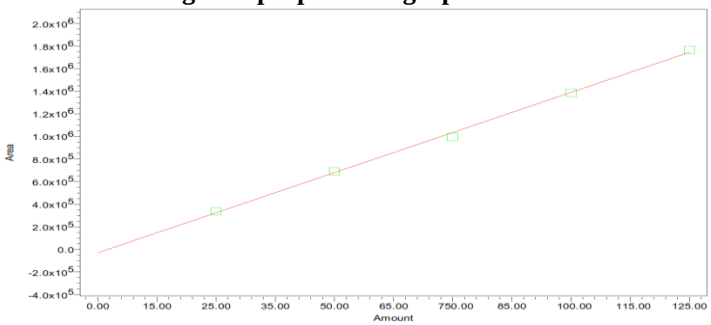
Linearity Results for Ornidazole:

Showing calibproportionn graph for Povidone



Povidone r2= 0.999

Showing calibproportionn graph for Ornidazole



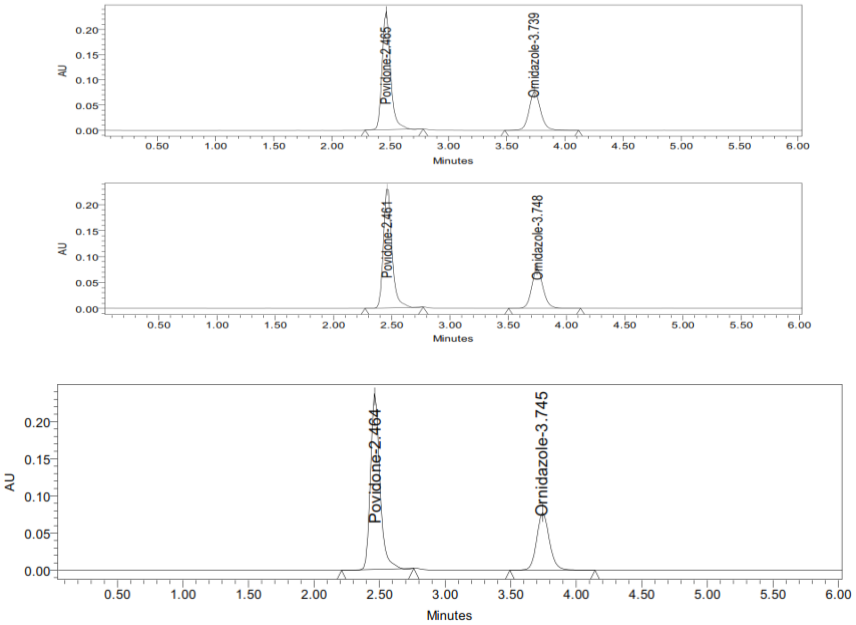
Ornidazoler^2 = 0.999

The linearity study were performed for concentproportionn range of 10µg-50µg Povidone and 25µg - 125 µg Ornidazole and the correlation coefficient were found to be 0.999 and 0.999.(NLT 0.999)individually.

Accuracy:

The accuracy study were performed for 50%, 100% and 150 % for Povidone andOrnidazole. Each level were injected in triplicate into chromatographic system. The area of each level were used for calculation of % recovery. Chromatograms are shown below

Chromatograms showingaccuracy-50% injection-1,2,3



Peak Name: Povidone

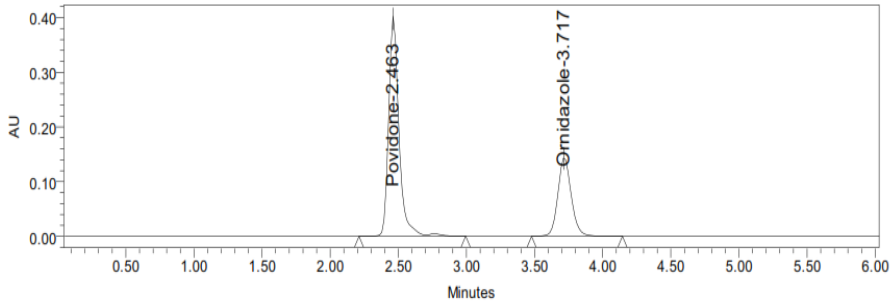
	Peak Name	RT	Area (µV*sec)	Height (µV)
1	Povidone	2.465	1182724	231695
2	Povidone	2.461	1183571	230394
3	Povidone	2.464	1186318	233578
Mean			1184204.3	
Std. Dev.			1878.6	
% RSD			0.2	

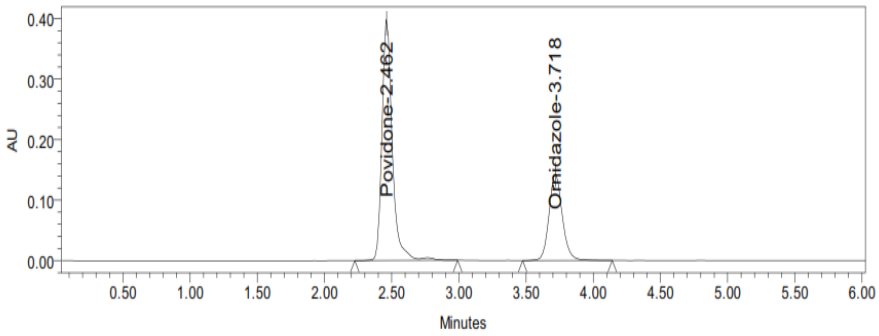
Peak Name: Ornidazole

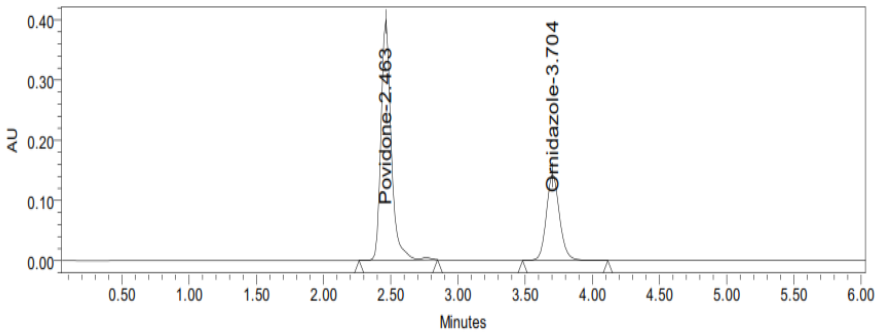
	Peak Name	RT	Area (µV*sec)	Height (µV)
1	Ornidazole	3.748	521676	76561
2	Ornidazole	3.739	521949	76731
3	Ornidazole	3.745	523030	77217
Mean			522218.2	
Std. Dev.			715.9	
% RSD			0.1	

Accuracy -100%

Chromatogram showing accuracy -100%injection-1,2,3.







Peak Name: Povidone

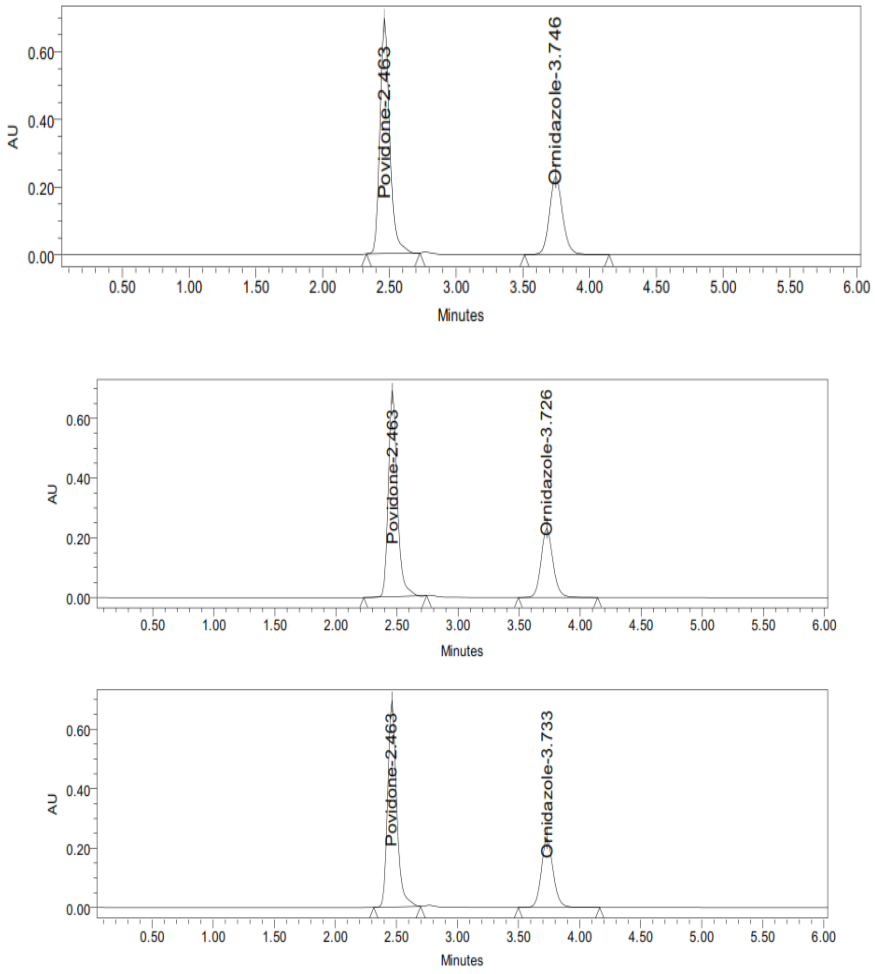
	Peak Name	RT	Area (µV*sec)	Height (µV)
1	Povidone	2.463	2113673	395149
2	Povidone	2.462	2122445	395053
3	Povidone	2.463	2129499	396470
Mean			2121872.4	
Std. Dev.			7928.3	
% RSD			0.4	

Peak Name: Ornidazole

	Peak Name	RT	Area (µV*sec)	Height (µV)
1	Ornidazole	3.718	977779	142756
2	Ornidazole	3.704	979030	143446
3	Ornidazole	3.717	981150	143439
Mean			979319.6	
Std. Dev.			1703.8	
% RSD			0.2	

Accuracy 150%

Chromatogram showing accuracy -150 %injection-1,2,3.



Peak Name:Povidone

	Peak Name	RT	Area (µV*sec)	Height (µV)
1	Povidone	2.463	3508712	681734
2	Povidone	2.463	3519906	684779
3	Povidone	2.463	3548681	685112
Mean			3525766.1	
Std. Dev.			20618.8	
% RSD			0.6	

Peak Name: ornidazole

	Peak Name	RT	Area (µV*sec)	Height (µV)
1	ornidazole	3.726	1570240	231207
2	ornidazole	3.746	1578806	231732
3	ornidazole	3.733	1580909	232561
Mean			1576651.8	
Std. Dev.			5651.3	
% RSD			0.4	

Showing accuracy results for Povidone

%Concentproportionn (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	1184204.3	5	4.98	99.93%	99.76%
100%	2121872.4	10	9.88	99.08%	
150%	3525766.1	15	15.0	100.00%	

Showing accuracy results for Ornidazole

%Concentproportionn (at specification level)	Average Area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	522218	5	4.99	99.93%	99.97%
100%	4551234	10	10.05	99.98%	
150%	5676042	15	14.95	99.56%	

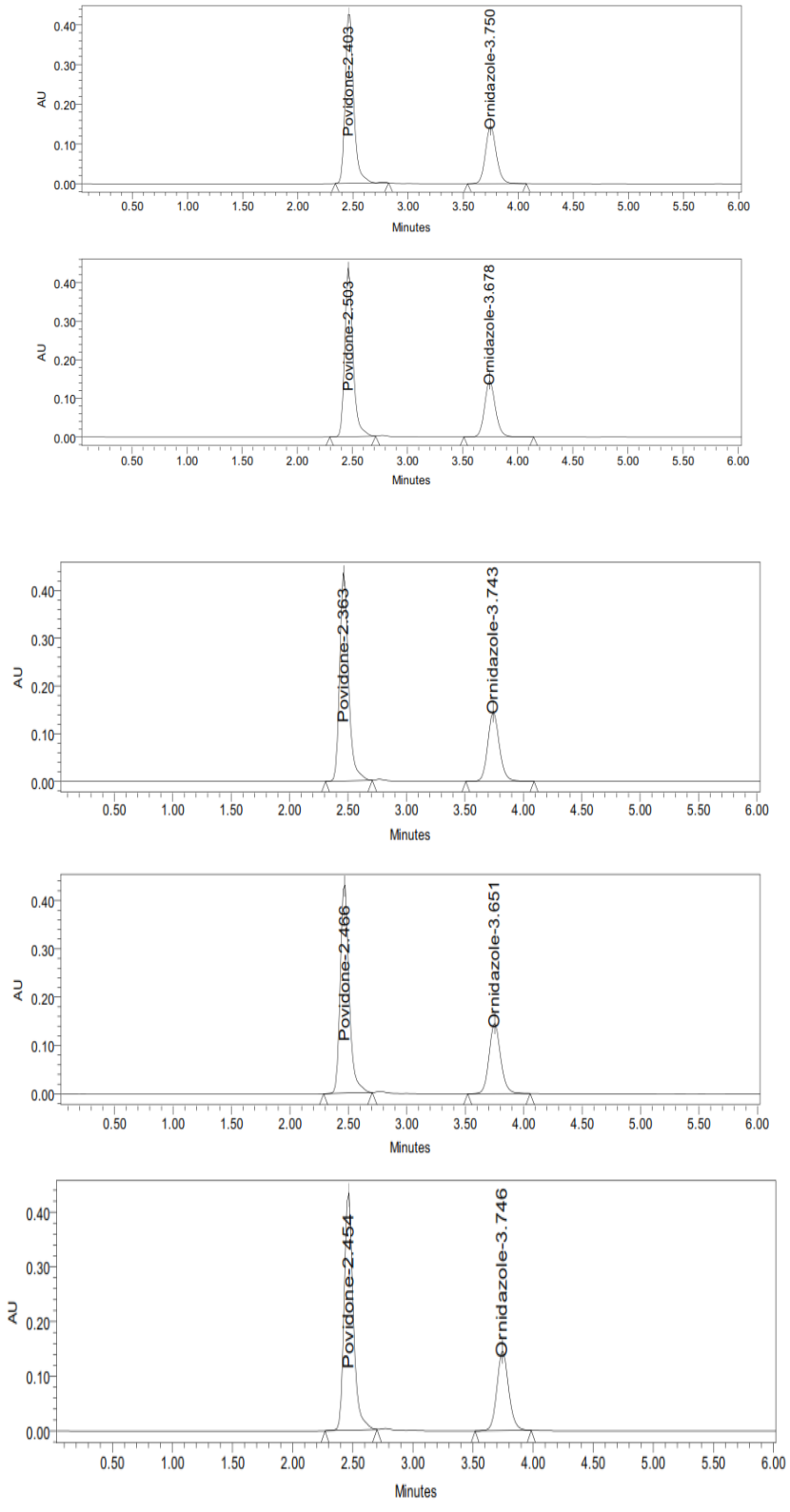
The accuracy study were performed for % recovery of Povidone and Ornidazole. The % recovery were found to be 99.96% and 99.97% individually (NLT 98% and NMT 102%)

Precision

Repeatability

The precision study were performed for five injections of Povidone and Ornidazole. Each standard injection were injected into chromatographic system. The area of each Standard injection were used for calculation of % RSD. The chromatograms are shown in below

Chromatograms showing precisioninjections-1 to 5



Showing% RSD results for Povidone

Peak Name:Povidone

	Peak Name	RT	Area (µV*sec)	Height (µV)
1	Povidone	2.403	1970553	432042
2	Povidone	2.503	1978100	433096
3	Povidone	2.363	1982356	428401
4	Povidone	2.466	1973157	429681
5	Povidone	2.454	1985975	431301
Mean			1978028.2	
Std. Dev.			6356.0	
% RSD			0.3	

Showing %RSD results for Ornidazole

Peak Name: Ornidazole

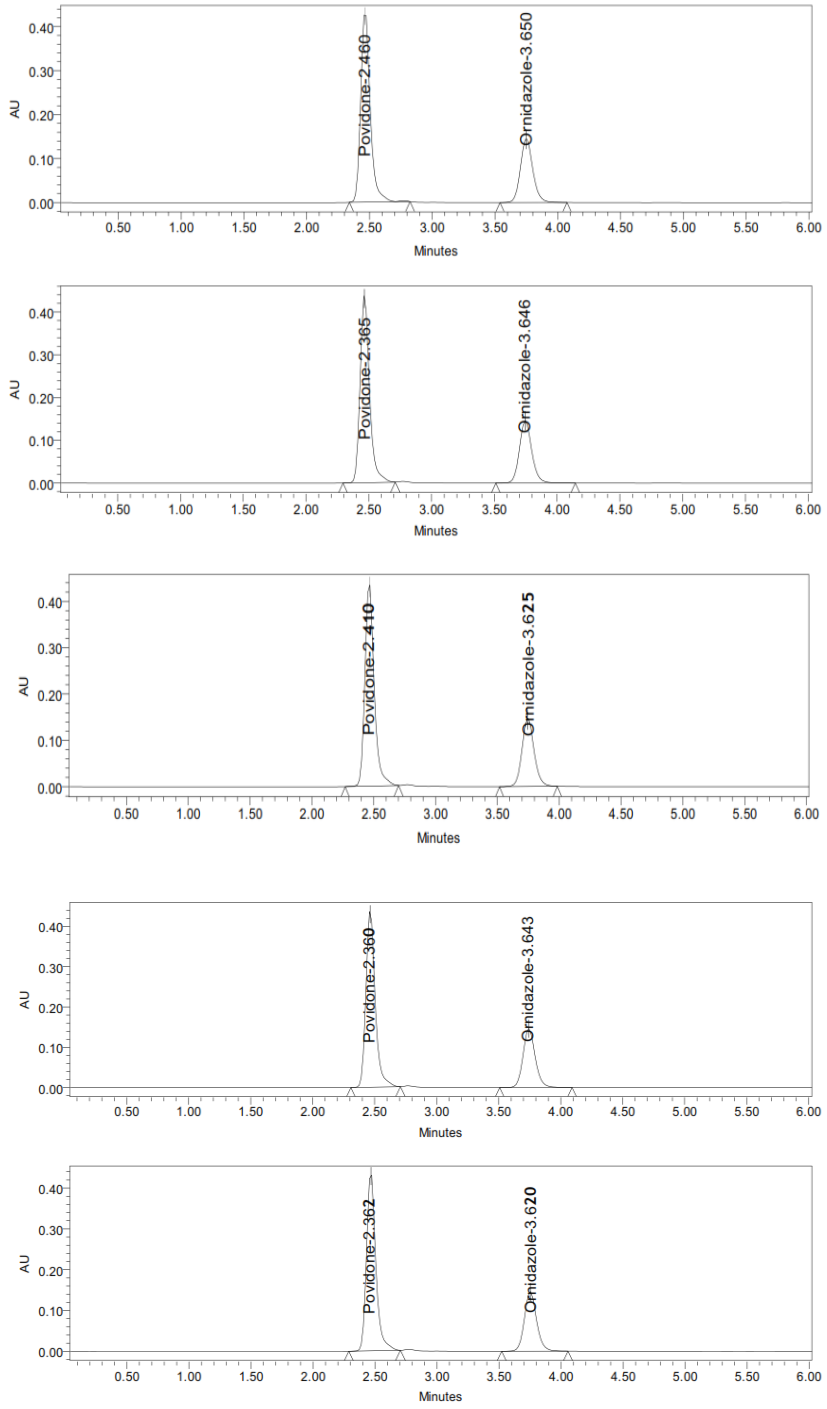
	Peak Name	RT	Area (µV*sec)	Height (µV)
1	Ornidazole	3.750	983413	145487
2	Ornidazole	3.678	985859	144337
3	Ornidazole	3.743	998213	145394
4	Ornidazole	3.651	988930	145762
5	Ornidazole	3.746	999663	145945
Mean			99121.6	
Std. Dev.			7333.4	
% RSD			0.7	

The Method precision studywere performed for the %RSD of Povidone and Ornidazole were found to be 0.3 and 0.7(NMT 2).

Intermediate precision/Ruggedness

The intermediate precision study were performed for five injections of Povidone and Ornidazole. Each standard injection were injected into chromatographic system. The area of each standard injection were used for calculation of % RSD.The chromatograms are shown in below

Chromatogramsshowingintermediate precision injections-1to 5



Showing results for intermediate precision of Povidone

Peak Name:Povidone

	Peak Name	RT	Area (µV*sec)	Height (µV)
1	Povidone	2.460	3270553	402042
2	Povidone	2.365	3178100	423096
3	Povidone	2.360	3282356	408401
4	Povidone	2.362	3283157	439681
5	Povidone	2.410	3285975	431301
Mean			2280028.2	
Std. Dev.			6001.7	
% RSD			0.3	

Showing results for intermediate precision of Ornidazole

Peak Name: Ornidazole

	Peak Name	RT	Area (µV*sec)	Height (µV)
1	Ornidazole	3.650	693413	245487
2	Ornidazole	3.646	683859	244337
3	Ornidazole	3.643	688213	204539
4	Ornidazole	3.620	698930	245762
5	Ornidazole	3.625	699663	245945
Mean			692815	
Std. Dev.			6819	
% RSD			0.9	

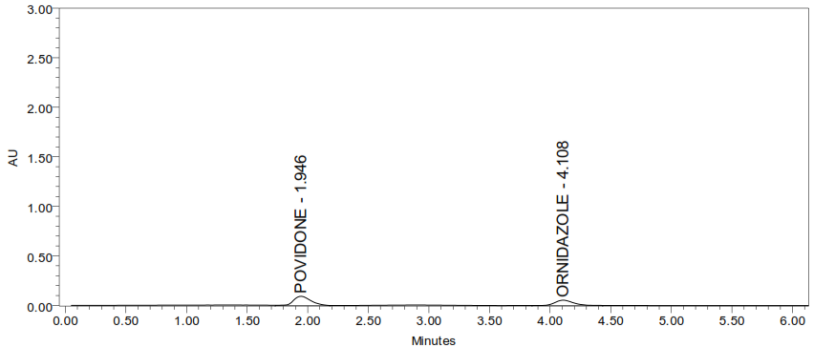
The intermediate precision were performed for %RSD of Povidone and Ornidazole were found to be 0.3 and 0.9individually (NMT 2).

Identification limit:

LOD's can be calculated based on the standard deviation of the response (SD)and the slope of the calibproportionn curve (S) at levels approximating the LOD according to the formula.The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Formula: LOD $= 3.3$ X $\frac{\sigma}{S}$

Where :σ - Standard deviation (SD) S - Slope



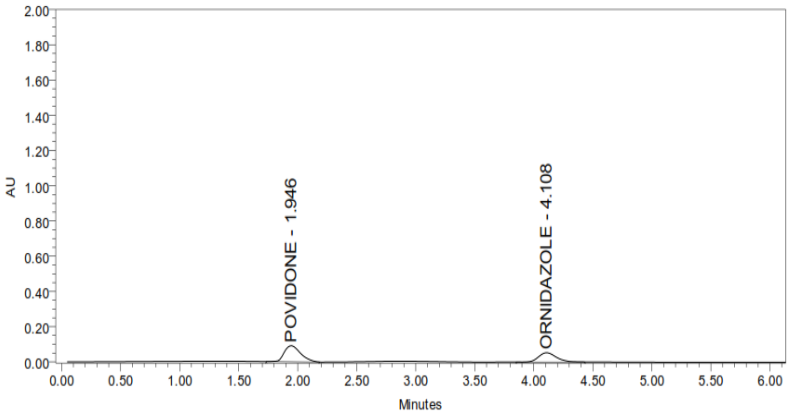
The LOD were performed for Povidone and Ornidazole were found to be 3.17and 0.2372individually.

Quantitation limit:

LOQ's can be calculated based on the standard deviation of the response (SD) and the slope of the calibproportionn curve (S) according to the formula. Again, the standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Formula:LOQ $= 10$ X $\frac{\sigma}{s}$

Where σ - Standard deviation S - Slope

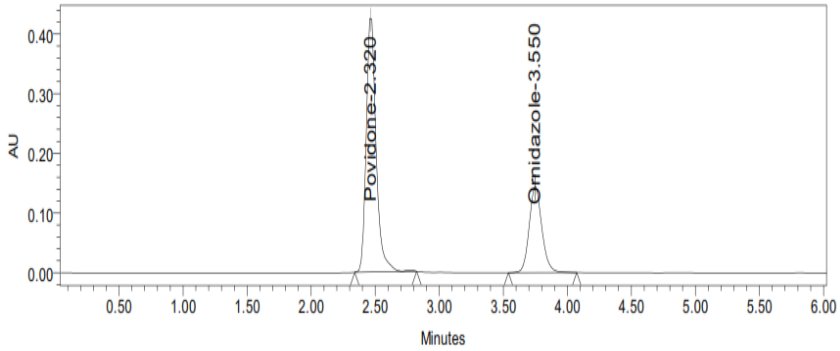


The LOQ were performed for Povidone and Ornidazole were found to be 7.80 and 5.30individually.

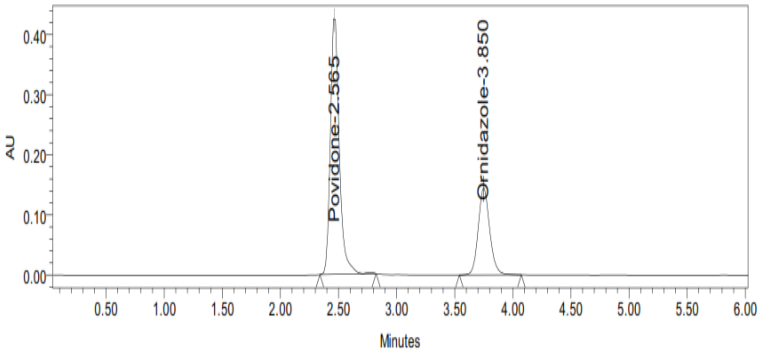
Robustness:

The robustness were performed for the flow rate variations from 0.8ml/min to 1.0 ml/min and mobile phase proportion variation from more organic phase to less organic phase proportion for Povidone and Ornidazole. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The chromatograms are shown in below

Chromatogram showing more flow rate 1.2ml/min



Chromatogram showing less flow rate 0.8 ml/min



The results are summarized on evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±0.2ml/min. The method is robust only in less flow condition.

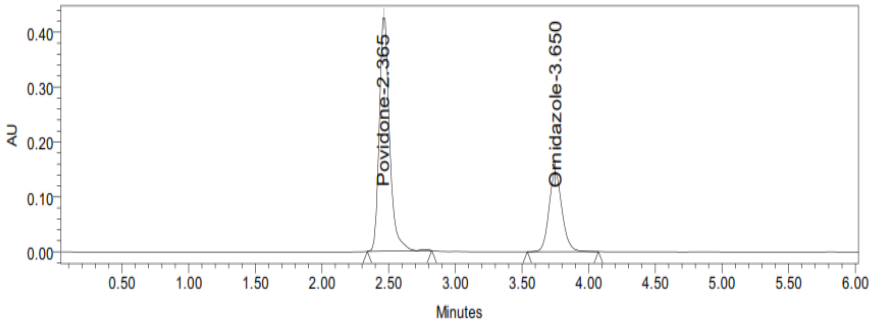
Showing system suitability results for Povidone

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	5568	1.2
2	**1**	**5358**	**1.2**
3	1.2	5210	1.2

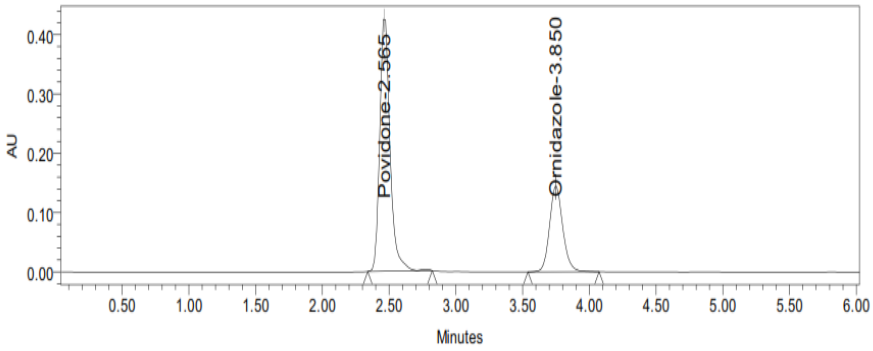
Showing system suitability results for Ornidazole

S.No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	7231	1.1
2	**1**	**7597**	**1.1**
3	1.2	7013	1.1

Chromatogram showing more organic phase proportion



Chromatogram showing less organic phase proportion



On evaluation of the above results, it can be concluded that the variation in±5%. Organic composition in the mobile phase affected the method significantly. Hence it indicates that the method is robust even by change in the mobile phase ±5%.

Showing system suitability results for Povidone

S.No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	5324	1.2
2	***Actual**	**5453**	**1.2**
3	5 % more	5123	1.1

Showing system suitability results for Ornidazole

S.No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	7595	1.1
2	***Actual**	**7465**	**1.1**
3	5 % more	7365	1.1

CONCLUSION:

A new method was established for simultaneous estimation of Povidone and Ornidazole by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Povidone and Ornidazole by using Inertsil C18 column (4.6×150mm)5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol: Phosphate buffer, detection wavelength was 240 nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The retention times were found to be 2.462 mins and 3.737 mins. The % purity of Povidone and Ornidazole was found to be 99.84% and 100.27% respectively. The system suitability parameters for Povidone and Ornidazole such as theoretical plates and tailing factor were found to be 5358.3, 1.2 and 7597 and 1.1, the resolution was found to be 8.4. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Povidone and Ornidazole was found in concentration range of 10µg-50µg and 25µg-125µg and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 99.96% and 99.98%, %RSD for repeatability was 0.3 and 0.7, % RSD for intermediate precision was 0.3 and 0.9 respectively. The precision study was precision, robustness and repeatabilty.LOD value was 3.17 and 0.2372 and LOQ value was 7.80 and 5.30 respectively.

Hence the suggested RP-HPLC method can be used for routine analysis of Povidone and Ornidazole in API and Pharmaceutical dosage form.

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