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A Validated RP-HPLC Method for Simultaneous Estimation of Indapamide Impurity (Methyl Nitrosoindoline) API form

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Abstract : A simple and accurate methods to determine Indapamide, in pure powder form, were developed and validated using liquid chromatography (LC). The LC separation was achieved on a Inertsil ODS 3V, 5µm, 150 x 4.6 mm,5µ in the isocratic mode using Mixture of 7 volume of acetoinitrile, 20 volume of Tetrahydrofuran and 73 volumes of a 1.5g/l solution of Triethylamine adjusted pH 2.8 with ortho-phosphoric acid at a flow rate of 1.4 ml/min. the methods were performed at 305 nm; In LC method, quantification was achieved with PDA detection over the concentration range of 80 to 120 µg/ml. The methods were validated, and the results were compared statistically. They were found to be simple, accurate, precise, and specific. The methods were successfully applied for the determination of Indapamide in pure powder form without any interference from common excipients.
Key words: Indapamide (Figure :3), PDA detection, Liquid Chromatography

Introduction

Indapamide is an oral antihypertensive/diuretic. Its molecule contains both a polar sulfamoyl chlorobenzamide moiety and a lipid- soluble methylindoline moiety. It differs chemically from the thiazides in that it does not possess the thiazide ring system and contains only one sulfonamide group. The chemical name of indapamide is 4-Chloro-N- (2-methyl-1- indolinyl) -3-Sulfamoylbenzamide, and its molecular weight is 365. 84. The compound is a weak acid and soluble in aqueous solutions of strong bases. It is a white to yellow-white crystalline powder.

Each tablet, for oral administration, contains indapamide 1.25 mg or 2.5 mg. In addition, each tablet contains the following inactive ingredients: FD&C Yellow No. 6 (1.25 mg), hydroxypropyl methylcellulose, lactose monohydrate, magnesium stearate, maize starch, microcrystalline cellulose, polyethylene glycol, povidone and titanium dioxide.

Indapamide is the first of a new class of antihypertensive/diuretics,[1] the indolines. It has been reported that the oral administration of 2.5 mg (two 1.25 mg tablets) of indapamide to male subjects produced peak concentrations of approximately 115 mg/ml of the drug in blood within two hours. It has been reported that the oral administration of 5 mg (two 2.5 mg tablets) of indapamide to healthy male subjects produced peak concentrations of approximately 260 mg/ml of the drug in the blood within two hours. A minimum of 70% of a single oral dose is eliminated by the kidneys and an additional 23% by the gastrointestinal tract, probably including the biliary route. The half-life of indapamide in whole blood is approximately 14 hours. Indapamide is preferentially and reversibly taken up by the erythrocytes in the peripheral blood. The whole blood/plasma [2] ratio is approximately 6:1 at the time of peak concentration and decreases to 3.5:1 at eight hours. From 71 to 79% of the indapamide in plasma is reversibly bound to plasma

proteins. Indapamide is an extensively metabolized drug with only about 7% of the total dose administered, recovered in the urine as unchanged drug during the first 48 hours after administration. The urinary elimination of ^{14}C-labeled indapamide and metabolites[3,4,5,6] is biphasic with a terminal half-life of excretion of total radioactivity of 26 hours.

In a parallel design double-blind, placebo controlled trial in hypertension,[7] daily doses of indapamide between 1.25 mg and 10 mg produced dose-related antihypertensive effects.[4] Doses of 5 and 10 mg were not distinguishable from each other although each was differentiated from placebo and 1.25 mg indapamide. At daily doses of 1.25 mg, 5 mg and 10 mg, a mean decrease of serum potassium of 0.28, 0.61 and 0.76 mg/ ml, respectively, were observed and uric acid increased by about 0.69 mg/100 ml. In other parallel design, dose-ranging clinical trials in hypertension and edema, daily doses of indapamide between 0.5 and 5 mg produced dose-related effects. Generally, doses of 2.5 and 5 mg were not distinguishable from each other although each was differentiated from placebo and from 0.5 or 1 mg indapamide.[8] At daily doses of 2.5 and 5 mg a mean decrease of serum potassium of 0.5 and 0.6 mg/Liter, respectively, was observed and uric acid increased by about 1 mg/100 ml.[9] At these doses, the effects of indapamide on blood pressure and edema are approximately equal to those obtained with conventional doses of other antihypertensive/diuretics.[10,11]
In a small number of controlled studies, indapamide taken with other antihypertensive drugs such as hydralazine, propranolol, guanethidine and methyldopa, appeared to have the additive effect typical of thiazide type diuretics.

Dosage and administration

Hypertension [7]: The adult starting indapamide dose for hypertension is 1.25 mg as a single daily dose taken in the morning. If the response to 1.25 mg is not satisfactory after four weeks, the daily dose may be increased to 2.5 mg taken once daily. If the response to 2.5 mg is not satisfactory after four weeks, the daily dose may be increased to 5 mg taken once daily, but adding another antihypertensive should be considered.

Edema of congestive heart failure: The adult starting indapamide dose for edema of congestive heart failure is 2.5 mg as a single daily dose taken in the morning. If the response to 2.5 mg is not satisfactory after one week, the daily dose may be increased to 5 mg taken once daily. If the antihypertensive response to indapamide is insufficient, indapamide may be combined with other antihypertensive drugs, with careful monitoring of blood pressure. It is recommended that the usual dose of other agents be reduced by 50% during initial combination therapy. As the blood pressure response becomes evident, further dosage adjustments may be necessary.

In general, doses of 5 mg and larger have not appeared to provide additional effects on blood pressure or heart failure, but are associated with a greater degree of hypokalemia. There is minimal clinical trial experience in patients with doses greater than 5 mg once a day.

Adverse effects

Commonly reported adverse events are hypokalemia (low potassium levels), fatigue, orthostatic hypotension (blood pressure decrease on standing up) and allergic manifestations.

Experimental

Apparatus

A Shimadzu Corporation HPLC Class-VP, instrument equipped with PDA detector SPD-10-AVP, Auto injector of SIL-10-ADVP and Inertsil ODS 3V, 150 x 4.6 mm, 5µ was used. A Sartorious BP-110S (Gottingen, Germany) analytical balance, and an ultra sonic cleaner (Electrolab) were also used.

Reagents and materials

Indapamide working standard (Potency : 100%), and Methylnitrosoindoline (Potency : 98.33%) Torrent Research Centre, (Ahmedabad, India) were given a gift sample. And provide a HPLC facility. HPLC grade acetonitrile were purchased from SDfine chemical (Ahmedabad, India). The water for LC was prepared by triple glass distillation and filtered through nylon 0.45µm-47mm membrane filter (Gelman laboratory, Mumbai, India). Tetrahydrofuran, orthophosphoric acid and triethylamine were procured from SD fine chemical (Ahmedabad, India) and were of analytical grade.

Chromatographic conditions

LC method

Inertsil ODS 3V, 150 x 4.6 mm, 5µ, in the isocratic mode using Mixture of 7 volume of acetoinitrile, 20 volume of Tetrahydrofuran and 73 volumes of a 1.5g/l solution of Triethylamine adjusted pH 2.8 with ortho-phosphoric acid. at a flow rate of 1.4 ml/min. The mobile phase was filtered through nylon 0.45µm-47mm membrane filter and was degassed before use. The elution was monitored at 305nm, and the injection volume was 30µL. Oven temp is 30ºC and sample cooler temp is 4ºC.

Preparation of mobile phase

Mixture of 7 volume of acetoinitrile, 20 volume of Tetrahydrofuran and 73 volumes of a 1.5g/l solution of Triethylamine adjusted pH 2.8 with ortho-phosphoric acid.

Preparation of diluent

Water: ACN (10:90)

(a) LC method

Preparation of Standard solutions

1. Dilute 2ml of 0.125 mg/L solution of methyl nitrosoindoline standard in acetonitrile to 25ml with diluents. (For RT conformation) (10 ppm)
2. Dilute 4ml of 0.125 mg/L solution of methyl nitrosoindoline standard in acetonitrile to 25ml with diluents. (For replicate preparation) (20 ppm) (Figure : 1)

Preparation of Sample solutions

Crush not less than 20 tablets in a motor and sieve through # 40 mesh. Transfer the remaining on the sieve to motor, crush and sieve. Repeat the process of crushing and sieving till nothing is left on the sieve. Mix the powder well. Weight accurately sample powder equivalent to about 25mg of Indapamide into a 25ml volumetric flask, add about 10ml diluents and shake for 15 minutes and make up the volume with diluents, mix. Allow standing at 4 ° C for 1 hour and filtering through 0.45µm filter and inject. (Inject freshly preparation only).

Method validation

Specificity

Interference from blank, placebo and Impurities

A blank, placebo preparation for Indapamide SR 1.5 mg tablet, sample preparation, individual impurity solution, and sample preparation spiked with Methyl nirosoindoline impurity (at 200 ppm with respect to test concentration) were prepared and injected. The peak purity index for the main peak and impurity peak in sample preparation and the sample preparation spiked with Methylnitrosoindoline impurity were determine and shown in Table -1.

Check for interference from forced degradation study

The placebo of Indapamide SR 1.5 mg, Indapamide API and Indapamide SR 1.5 mg were subjected to acid, base, and oxidation, thermal. For each degradation, a blank were prepared separately and ensure that for at least two conditions degradation is achieved.

A blank, stressed placebo of Indapamide SR 1.5 mg, Stressed Indapamide API and stressed Indapamide SR 1.5 mg tablets sample solutions were prepared and injected. The peak purity index for the main peak and degraded known impurity peak in all the degraded sample preparation was determined and shown in Table-2,3,4,5.

Calibration curve (Linearity)

[B]) LC method

Linearity was determined at six levels over the range of (LOQ) to 150% of specification limit with respect to the concentration of sample preparation. Each standard preparation was injected in duplicate. The mean area at each level was calculated and a graph of mean area versus concentration was plotted. The correlation coefficient (r), y-intercept, slope of regression line and residual sum of squares were calculated.

30µL of each solution were injected under the operating chromatographic conditions as described above. Calibration curves were constructed by plotting peak areas vs. concentrations of Indapamide, and the regression equations were calculated. Each reading was average of three determinations values.

Method precision (Repeatability)

Method precision was established by calculating related impurities in six sample preparations. Known impurities in ppm, SD, %RSD and 95% confidence interval were calculated. (Figure : 2)

Intermediate precision

The procedure followed for method precision was repeated on a different day, by a different analyst, using a different column and HPLC system, known impurities in ppm, SD, %RSD and 95% confidence interval were calculated. The difference of the impurity values obtained in the Intermediate precision study and the same obtained in Method precision study was calculated.

Robustness

The robustness of the method was established by making deliberate minor variations in the following method parameters.

(1) Changed the flow rate by ±0.1 ml/min. (i.e. 1.3 ml/min. and 1.5 ml/min.)
(2) Changed the minor component (Acetonitrile) in the mobile phase by ±2 %absolute.
(3) Changed the column temperature by ±5ºC. (i.e. 25ºC & 35ºC)
(4) Changed in buffer pH ± by 0.2 units. (i.e. pH- 2.6 and 3.0)
(5) Changed in column lot.

Blank, diluted standard preparation and the sample preparation were prepared and injected.

Limit of Detection and Limit of Quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were calculated using the following equations as per International Conference on Harmonization (ICH) guidelines (41).

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where σ = the standard deviation of the response

S= the slope of the regression line

Stability in analytical solution

Prepared and analysed blank preparation, reference solution, sample preparation as per method. Diluted standard preparation and sample preparation were stored up to 48 hours at room temperature and at 4ºC (sample cool rack) and analysed up to 48 hours against freshly prepared reference solution. Establish the solution stability period.

Accuracy (% Recovery)

The accuracy of the method was established at three levels in the range of LOQ-150% of specification

limit. Calculated amount of known impurity were added in placebo to attain LOQ, 50%, 100% and 150% of specification limit. Triplicate preparations were prepared as described in protocol, for each level and each preparation was injected in duplicate. % Recovery, mean % recovery and %RSD was calculated at each level and recorded in Table-8.

Results and discussion

(a) LC method

To optimize the LC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for Indapamide was obtained with mobile phase consisting of Mixture of 7 volume of acetoinitrile, 20 volume of Tetrahydrofuran and 73 volumes of a 1.5g/l solution of Triethylamine adjusted pH 2.8 with ortho-phosphoric acid. to obtain better reproducibility and repeatability. Quantification was achieved with PDA detection at 280 nm based on peak area. Better resolution of the peaks with clear base line separation was found.

Validation of the proposed methods

Linearity

Linear correlation was obtained between peak area and concentration of Indapamide in the range of 50.0 to 150.00µg/ml for LC method. The linearity of the calibration curves was validated by the value of correlation coefficients of the regression (r = 0.9993).

Method Precision

The percentage relative standard deviation (% RSD) for Indapamide was found to be 4.77 % by LC method. The RSD values indicate the proposed methods are repeatable.

Accracy(Recovery)

(1) Individual recovery at LOQ level and mean recovery should be in the range of 85.0 to 115.0% and RSD should not be mere than 10.0%.
(2) Individual recovery other than LOQ level and mean recovery other than LOQ level should be in the range of 90.0 to 110.0% and RSD should not be more than 10.0%.
(3) The recovery (individual) and %Mean recovery at each level meets the established acceptance criteria. Hence the method can be termed accurate in the considered range.

Intermediate Precision (Reproducibility)

The percentage relative standard deviation (% RSD) for Indapamide was found to be 4.54 % respectively, by LC method. The RSD values indicate the proposed methods are repeatable

Solution stability

The solution stability was checked for sample preparation up to 48 hours. The results obtained are well within the acceptance criteria up to 24 hours at room temperature & at 4ºC. Therefore, the sample preparation is stable in solution from up to 24 hours at room temperature & 4ºC.Results were determined and record in table-9.

System suitability

The difference in the known impurity value of individual preparation should not be more than 10% absolute from the mean value.

The data indicate that there is no significant difference between the results obtained under normal condition and varied method parameters. Therefore method is robust.

Specificity

There is no interference of blank, placebo and impurity peaks with the main peak. The purity index obtained is well within the limit of acceptance criteria. The known impurity is separated from main peak. The method can therefore be termed as specific.

The peak purity index results lies well within the acceptance criteria. Therefore, the method can be termed as specific under stress conditions.

Peak purity for the analyte peak & known impurity peak in standard preparation, sample preparation and degraded sample preparation should be more than 0.99

(1) There should not be any interference from blank & placebo peaks with impurity peaks and main peak.
(2) Peak purity for the main peak and impurity peak in sample preparation and sample spiked with impurity preparation should not be less than 0.99.

Limit of detection and limit of quantification

The LOD and LOQ of indapamide were found to be 0.0003 and 0.0009 µg/ml, respectively by LC. The validation parameters are summarized in Table 6. The proposed method is found to be specific, as no interference of excipients was found in the estimation of indapamide in tablet dosage form.

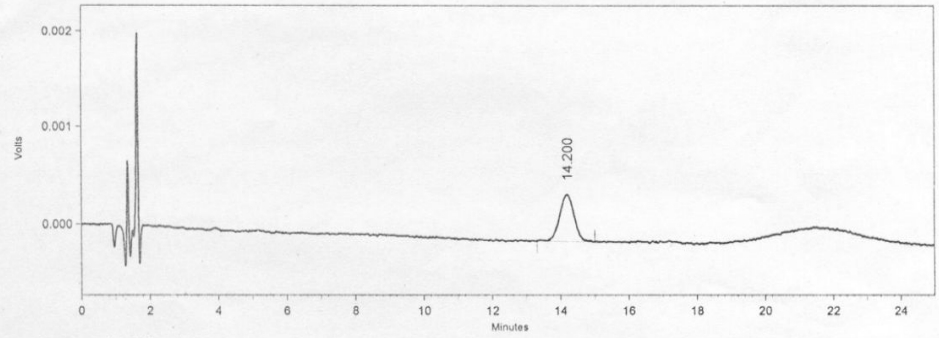
Robustness

The data indicate that there is no significant difference between the results obtained under normal condition and varied method parameters. Therefore method is robust. And all data obtained table no 10.

Conclusion

The system suitability parameters are well within acceptance criteria. Therrefore, the system and chromatographic conditions were suitable during each validation parameter. Since the results are within acceptance criteria for all validation parameters. Therefore, the method is considered as validated and suitable for intended use. Also, the method is specific for Indapamide API in presence of blank, therefore the method is stability indicating.

Sample ID : Indapamide SR Tablet[D0071]
Method Name : E:\HPLC290\D0071\200207\RI.met
File Name : E:\HPLC290\D0071\200207\290-200207-02
Sequence Nam : E:\HPLC290\D0071\200207\RI.seq
Sample Description : Standard preparation-0.125 mg/Ltr
Method - As per EP.
Column; Inertsil ODs/150*4.6/5µm
Acquired Date : 20/02/2007 11:09:13
Injection Volume :20 ul



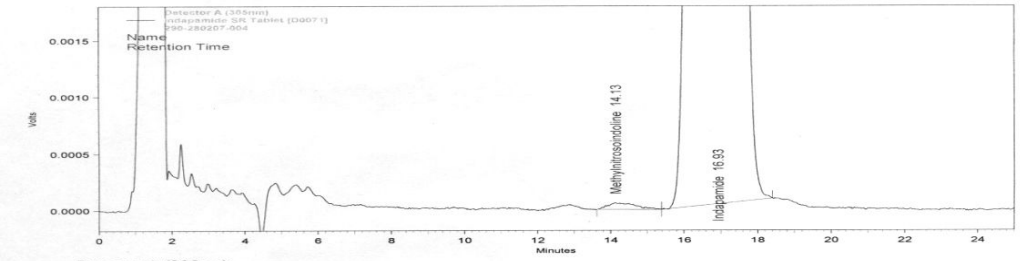
Detector A (305nm)

Pk #	Name	Retention Time	Area	Area Percent	Resolution
1	Methylnitrosoindoline	14.20	14321	100.00	0.00
Totals			14321	100.00	

Figure 1 : Chromatogram of indapamide standard preparation (0.125mg/ml)

Parameter: precision

Sample ID :Indapamide SR Tablet [D0071]
Method Name :E:\HPLC290\D0071\280207\Method Precision.met
File Name :E:\HPLC290\D0071\280207\290-280207-004
Sequence Name :E:\HPLC290\D0071\280207\290-280207.seq
Sample Description :Method Precision Set -1
B.No.B6546006
Conc-1000 ppm
Acquired Date :28/02/2007 13:28:22
Vial :10004
Injection Volume :30µl
User Name : Vipul



Detector A (305nm)

Pk #	Name	Retention Time	Area	Area Percent	Resolution
1	Methylnitrosoindoline	14.13	3121	0.03	0.00
2	Indapamide	16.93	10386879	99.97	2.11
Totals			10390000	100.00	

Figure 2 : Chromatogram of indapamide precision

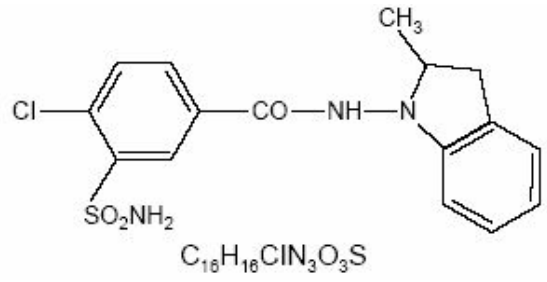


Figure 3 : Indapamide

Table 1 : Interference from blank, placebo and impurities

Solution	Peak purity index	
	Known impurity-Methylnitrosoindoline	**Indapamide**
Blank preparation	**-----**	**-----**
10 ppm standard preparation for Methylnitrosoindoline	**1.00000**	**-----**
200 ppm standard preparation for Methylnitrosoindoline	**1.00000**	**-----**
Placebo preparation	**-----**	**-----**
Sample 1.5 mg (as such)	**1.00000**	**1.00000**
Sample 1.5 mg (spiked)	**0.99981**	**1.00000**

Table 2 : Thermal degradation

Sample	ppm of Methylnitr-osoindoline	Peak purity index	
		Methylnitr-osoindoline	**Indapamide**
Blank	-----	-----	-----
Blank Acid base degradation	-----	------	-----
Placebo 1.5 mg Unstressed	-----	-----	-----
Placebo 1.5 mg stressed Thermal degradation	-----	-----	-----
Sample 1.5 mg Unstressed	26.77	1.00000	1.00000
Sample 1.5 mg stressed Thermal degradation	43.01	1.00000	1.00000
Indapamide API unstressed	36.80	-----	-----
Indapamide API stressed Thermal degradation	71.98	1.00000	1.00000

Table 3 : Acid degradation

Sample	ppm of Methylnitr-osoindoline	Peak purity index	
		Methylnitr-osoindoline	**Indapamide**
Blank	-----	-----	-----
Blank Acid base degradation	-----	------	-----
Placebo 1.5 mg Unstressed	-----	-----	-----
Placebo 1.5 mg stressed 0.1N HCl degradation	-----	-----	-----
Sample 1.5 mg Unstressed	26.77	0.99925	0.99999
Sample 1.5 mg stressed 0.1N HCl degradation	164.45	0.99561	1.00000
Indapamide API unstressed	36.80	1.00000	0.99999
Indapamide API stressed 0.1N HCl degradation	65.26	0.99256	1.00000

Table 4 : Base Degradation

Sample	ppm of Methylnitr-osoindoline	Peak purity index	
		Methylnitr-osoindoline	Indapamide
Blank	-----	-----	-----
Blank Acid base degradation	-----	------	-----
Placebo 1.5 mg Unstressed	-----	-----	-----
Placebo 1.5 mg stressed 0.1N NaoH degradation	-----	-----	-----
Sample 1.5 mg Unstressed	26.77	0.99925	0.99999
Sample 1.5 mg stressed 0.1N NaoH degradation	138.86	0.99587	1.00000
Indapamide API unstressed	36.80	1.00000	0.99999
Indapamide API stressed 0.1N NaoH degradation	47.55	0.99254	1.00000

Table 5 : Oxidation Degradation

Sample	ppm of Methylnitr-osoindoline	Peak purity index	
		Methylnitr-osoindoline	Indapamide
Blank	-----	-----	-----
Blank peroxide degradation	-----	------	-----
Placebo 1.5 mg Unstressed	-----	-----	-----
Placebo 1.5 mg stressed 10% H_2O_2 degradation	-----	-----	-----
Sample 1.5 mg Unstressed	26.77	0.99925	0.99999
Sample 1.5 mg stressed 10% H_2O_2 degradation	1142.97	0.99686	1.00000
Indapamide API unstressed	36.80	1.00000	0.99999
Indapamide API stressed 10% H_2O_2 degradation	291.51	0.99227	1.00000

As per statistical value of LOD & LOQ

Table 6 : Limit of detection & limit of quantitation

Level	Methylnitrosoindoline	
	Conc. (ppm)	Mean Area
Level 1	0.00125	Not detected
Level 2	0.00250	447
Level 3	0.00375	730
Level 4	0.00500	938
Level 5	0.00625	1228
Level 6	0.00750	1458
Correlation coefficient (r)	0.9959	
Slope of regression line	201600	
Residual sum of squares	-47.8	
LOD in ppm	0.0003	
LOQ in ppm	0.0009	

Table 7 : Linearity and Range

Sr. No.	% Linearity level	Methylnitrosoindoline	
		Conc. in ppm	Average area
1	LOQ	0.01	1716
2	50%	0.10	15979
3	75%	0.15	25302
4	100%	0.20	32516
5	125%	0.25	40522
6	150%	0.30	48479
Correlation coefficient (r)		0.9993	
Slope of regression line		161475	
y-intercept		+ 237.41	

Table 8 : Accuracy

Level	Amount found (mg/ml)	Amount added (mg/ml)	Recovery (%)	Mean (%)	%RSD
Level-(LOQ)	0.00001006	0.0001006	98.66	102.40	3.26
	0.0000105721	0.0001006	105.09		
	0.0000104071	0.0001006	103.45		
Level-1 (50%)	0.000132717	0.00012575	105.54	106.86	1.53
	0.000136678	0.00012575	108.69		
	0.000133735	0.00012575	106.35		
Level-2 (100%)	0.000251395	0.000252	99.76	99.22	0.48
	0.000249581	0.000252	99.04		
	0.000249127	0.000252	98.86		
Level-3 (150%)	0.000363541	0.000377	96.43	95.81	0.61
	0.000360902	0.000377	95.73		
	0.000359168	0.000377	95.27		

Table 9: System suitability

For sample preparation			
Time	**Conditions**	**Impurity in ppm**	**Absolute difference (ppm)**
(A) For Methylnitrosoindoline			
Initial	Room temperature	46.4	Not applicable
After 12 hours	Room temperature	41.8	4.6
After 24 hours	Room temperature	37.9	8.5
After 48 hours	Room temperature	34.0	12.4

(B) For Methylnitrosoindoline			
Initial	4ºC	46.4	Not applicable
After 12 hours	4ºC	43.1	3.3
After 24 hours	4ºC	38.0	8.4
After 48 hours	4ºC	31.6	14.8

Table 10 : Effect on Impurity results

Condition	Methylnitrosoindoline Impurity in ppm
(A) Change in flow rate	
Normal condition (1.4 ml/min.)	39.6
Change in flow rate by -0.1 ml/min. (1.3 ml/min.)	18.3
Absolute difference from Normal condition	21.3
Change in flow rate by +0.1 ml/min. (1.5 ml/min.)	17.6
Absolute difference from Normal condition	21.0
(B) Change in mobile phase composition	
Normal condition M.P.-Buffer : ACN : THF (73:07:20)	39.0
Change in ACN composition by -2% absolute Buffer : ACN : THF (75:05:20)	36.7
Absolute difference from normal condition	2.3
Change in ACN composition by +2% absolute Buffer : ACN : THF (71:09:20)	11.3
Absolute difference from normal condition	27.7
(C) Change in column temperature	
Normal condition (30ºC)	39.6
Change in oven temperature by -5ºC (25ºC)	16.3
Absolute difference from normal condition	23.3
Change in oven temperature by -5ºC (25ºC)	15.3
Absolute difference from normal condition	24.3
(D) Change in buffer pH	
Normal condition (pH – 2.8)	39.6
Change in buffer pH by -0.2 unit (pH – 2.6)	21.6
Absolute difference from normal condition	18.0
Change in buffer pH by +0.2 unit (pH – 3.0)	14.2
Absolute difference from normal condition	25.4
(E) Change in wavelength	
Change in wavelength by -5 nm	24.5

Absolute difference from normal condition	15.1
Change in wavelength by +5 nm	21.9
Absolute difference from normal condition	17.7
(F) Change in column lot	
Normal : INSIL/03V/150-5/29	37.9
Changed : INSIL/03V/150-5/10	42.6
Absolute difference from normal condition	4.7

Abbreviations: µg : Microgram, mL : Mililiter,µL : Microliter,SD : Standard deviation,RSD : Relative Standard Deviation.

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