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Determination of 3-Chloro-1,2-Propanediol content in Iohexol drug substance by using High-Performance Liquid Chromatography with refractive index detector

Sanni Babu Najana[1*], Hari Babu Bollikolla[1]

[1*]*Department of Chemistry, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur, Andhra Pradesh, India.*
Corresponding author E-mail: n.sannibabu@gmail.com

ABSTRACT
A highly sensitive method for determining impurity, such as 3-Chloro-1,2-propanediol in Iohexol drug substance using RP-LC, has been presented in the present paper. Quantification of 3-Chloro-1,2-propanediol content in Iohexol samples by HPLC with Refractive Index (RI) Detector. The separation, detection, and quantification of 3-Chloro-1,2-propanediol were determined using Symmetry shield reverse phase C18 (250x4.6mm, 5µm) column as the stationary phase. Column temperature maintained 25°C, Injection volume 30µL, and the Flow rate was 0.6 ml/min, sample cooler temperature ambient, and RID temperature 30°C. pH 4.65 phosphate buffer was used as the mobile phase. The method validation has been carried as per International Conference on Harmonization guidelines. The limit of quantitation (LOQ) was found 1.29 ppm for 3-Chloro-1,2-propanediol.

Keywords: *3-Chloro-1,2-propanediol, Iohexol, Refractive index detector, validation, and limit of quantitation.*

I. Introduction

Synthesis of drug substances often involves reactive reagents; hence, these reagents may be present in the final drug substances as impurities. Such chemically reactive impurities may have unwanted toxicities, including genotoxicity and carcinogenicity, and are controlled based on the maximum daily dose [1]. These limits generally fall at low µg/mL levels. HPLC, GC methods (or final drug substance methods) are suitable for their determination. Their applications are oriented towards the potential identification and quantitation of trace levels of impurities in drug substances [2].

The chemical name of Iohexol is 5-[acetyl(2,3-dihydroxy propyl)amino]-1-N,3-N-bis (2,3-dihydroxy propyl)-2,4,6-triiodobenzene-1,3-dicarboxamide.
Molecular formula $C_{19}H_{26}I_3N_3O_9$. It has a relative molecular mass of 821.138 g/mol. Iohexol is employed as a radiographic contrast agent. Iohexol is a water-soluble and non-ionic agent used in radiographic methods like nephroangiography, arthrography, myelography, and arteriography [3-5]. Iohexol has a very good pharmacological profile with outstanding clinical efficacy, very low toxicity, and negligible interference with the regular organism's functions.

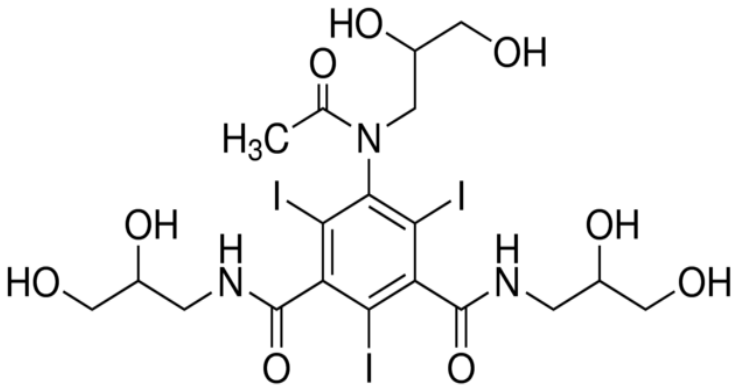

Figure: 1.0 Chemical structure of Iohexol

A. Impurity structure

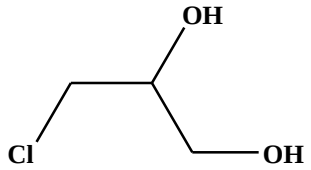

Figure: 2.0 Chemical structure of 3-Chloro-1,2-propanediol

In the manufacturing process of Iohexol, 3-Chloro-1,2-propanediol is used as a reagent. And hence 3-Chloro-1,2-propanediol may exist as an impurity in Iohexol drug substance.

The literature review showed that few analytical methods had been described to estimate 3-Chloro-1,2-propanediol, including gas chromatography methods (GC) [6-7] have been reported for the estimation of 3-Chloro-1,2-propanediol in Iohexol drug substance. No HPLC method was reported for the determination of 3-Chloro-1,2-propanediol in Iohexol. Hence, the author aimed to develop simple and cost-effective instrumentation, no need to take any special precautions during sample preparation, extraction free method, more repeatability and reproducibility, rapid, specific, and robust methods for determining the 3-Chloro-1,2-propanediol in Iohexol at trace level concentration.

II. Experimental

Chemicals and reagents

Potassium dihydrogen orthophosphate (AR Grade) was procured from Merck, India. 3-Chloro-1,2-propanediol was procured from Tokyo Chemical Industry Co. Ltd (TCI), Chennai, India. Water (Milli-Q system, Millipore). The drug substances of "Iohexol" for research obtained from Jodas Expoim Pvt. Ltd, Hyderabad, India.

Mobile phase:

Dissolved 5.44g of KH_2PO_4 in 1000 mL of Milli-Q-water and adjusted to pH 4.6±0.05 with ortho-phosphoric acid. Filter through 0.45µ membrane filter paper.

Preparation of diluent:

Milli Q water was used as diluent.

Chromatographic conditions

RP-LC analysis was carried out on the Agilent-1260 Infinity series (Agilent Corporation, USA). Symmetry shield reverse phase C18 (250x4.6mm, 5µm) column was used as the stationary phase. pH 4.60 phosphate buffer was used as the mobile phase. The flow rate of the mobile phase was kept at 0.6mL/min. The injection volume was set as 30µL. Column oven temperature 25°C and autosampler temperature ambient and refractive index detector was used. Refractive index detector temperature 30°C.

Preparation of standard stock solution (3-Chloro-1,2-propanediol):

Weighed accurately and transferred about 25mg of the standard into a 100ml volumetric flask, containing 50ml of diluent, which sonicated it to dissolve and make up to the volume with diluent.

Preparation of standard solution:

Transferred 2.0 ml of the standard stock solution into 100ml volumetric flask, containing 25ml of diluent sonicated it to dissolved and makeup to the volume with diluent. Further 5 ml of the standard solution into 50ml volumetric flask containing 25 ml of diluent mixed well and made up to the mark with diluent.

Preparation of Test solution:

Weighed accurately and transferred about 2000mg of test sample into a 10ml volumetric flask, containing 5ml of diluent, which sonicated it to dissolve and make up to the volume with diluent.

Preparation of sample spiked solution:

Weighed accurately and transferred about 2000mg of test sample into a 10ml volumetric flask. Dissolved in 5mL of diluent and added 20µL of 3-Chloro-1,2-propanediol standard stock solution. Mixed well and then made up to the mark with diluent.

III. Results And Discussions

A. Method development

A blend solution containing 3-chloro-1,2-propanediol and Iohexol was run in 1.0 mL/min flow rate. 3-chloro-1,2-propanediol eluted at void volume, and hence the flow rate of the mobile phase was decreased from 1.0 mL/min to 0.6 mL/min. In this condition, 3-chloro-1,2-propanediol eluted at an optimum retention time. The retention time of 3-chloro-1,2-propanediol was 9.680 min in standard solution, and 9.625 min in Iohexol sample solution spiked with 3-chloro-1,2-propanediol. Hence, the elution order was observed from the chromatograms (Figure no.3.0-6.0).

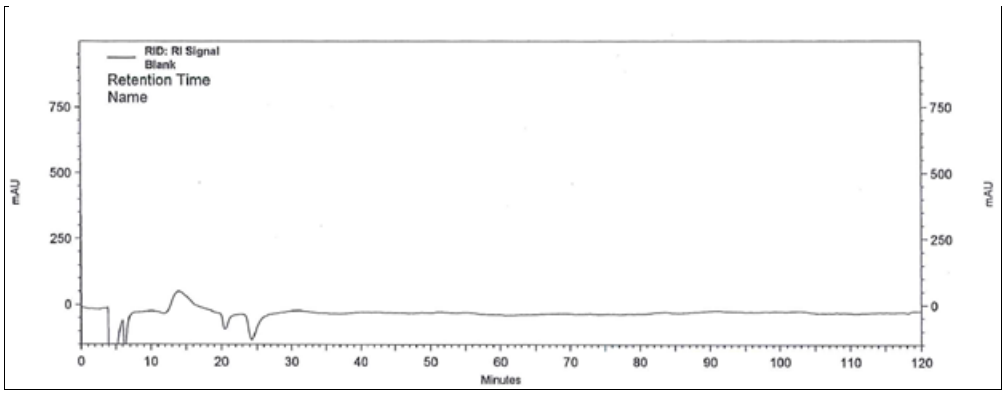

Figure: 3.0 typical chromatogram of Blank

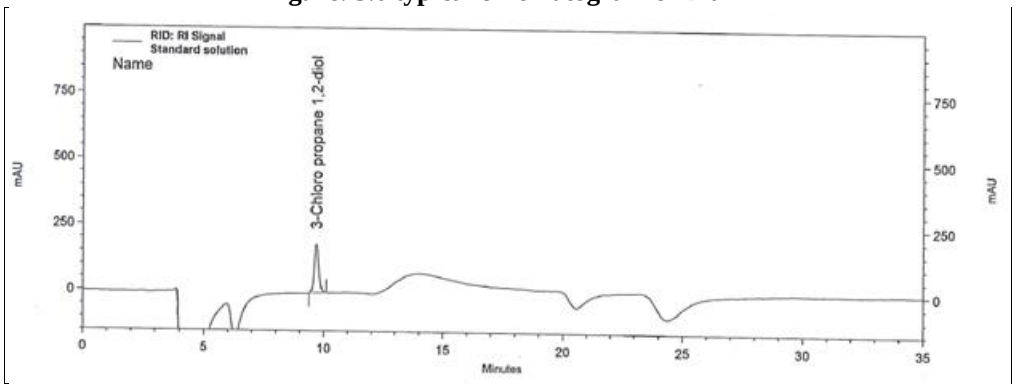

Figure: 4.0 typical chromatogram of standard

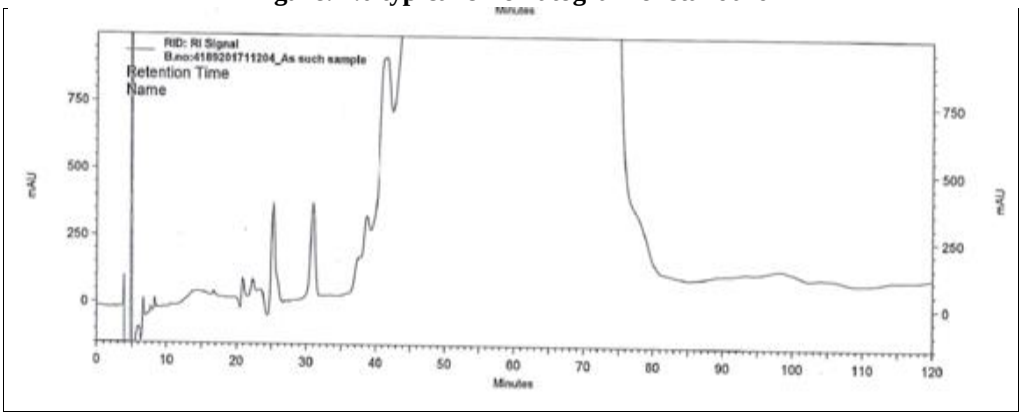

Figure: 5.0 typical chromatogram of the test sample

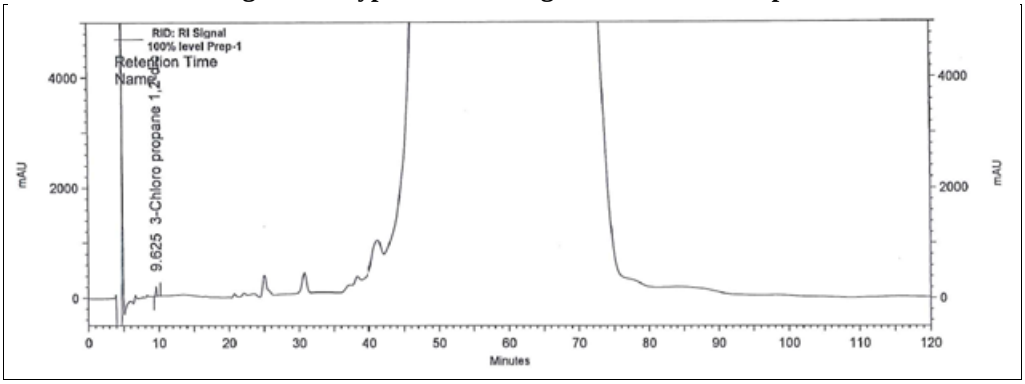

Figure: 6.0 Spiked 3-chloro-1,2-propanediol chromatogram of Iohexol

IV. Method validation

The method developed was validated in accordance with guidelines framed by the International Conference on Harmonization ICH [8].

A. Specificity

Blank interference

A study to establish the interference of blank was conducted. Diluent was injected as per the test method.

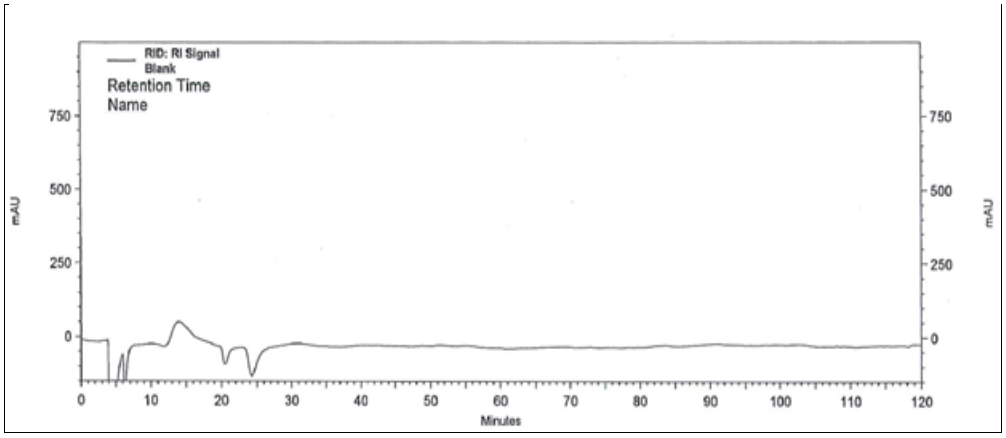

Figure: 7.0 typical chromatogram of Blank

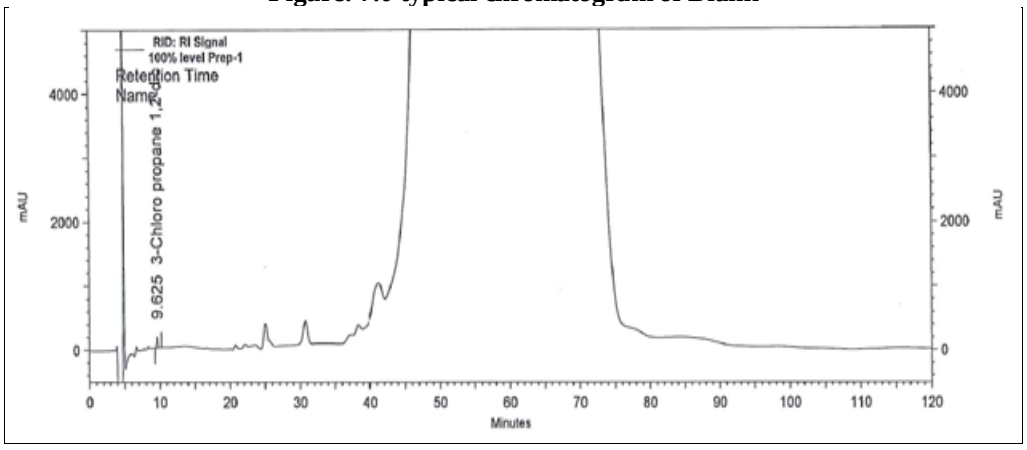

Figure: 8.0 Spiked 3-chloro-1,2-propanediol chromatogram of Iohexol

It was observed that the 3-chloro-1,2-propanediol peak was not co-eluting with the main analyte peak. Iohexol standard solution preparation and spiked test preparation were calculated and within the acceptable limit.

B. System suitability

Perform the reference solution analysis (Diluted standard) six times and determine the percentage relative standard deviation of peak area, tailing factor, and theoretical plates of replicate injections of 3-chloro-1,2-propanediol. The result of the system suitability study is tabulated in **Table: 1.0.**

Table 1.0 Results of system suitability for 3-chloro-1,2-propanediol

No. of Injections	Peak area	Tailing factor	Theoretical plates
1	239085	1.16	12942
2	246398	1.15	12926
3	238716	1.11	12949
4	239579	1.16	12792
5	237185	1.15	12869
6	237403	1.18	13034
Mean	**239728**	**1.152**	**12919**
%RSD	**1.42**	**1.01**	**0.63**

C. Method Precision

The impurity precision was determined by injecting six sample solutions spiked with impurities (3-chloro-1,2-propanediol) at the specification level. The samples were prepared as per the method, and the result for the precision study is tabulated in **Table: 2.0.**

Table: 2.0 Results of method precision

No. of Preparations	3-chloro-1,2-propanediol
1	212977
2	214360
3	213568
4	227845
5	214578
6	213659
Mean (%)	**216165**
% RSD	**2.66**

D. Limit of detection (LOD) & Limit of Quantitation (LOQ)

A solution containing 0.43 µg/ml of the 3-chloro-1,2-propanediol standard was injected three times. The worst found signal to noise ratio for each peak was greater than 3 in each injection. All the peaks were detected in all three injections.

Table: 3.0 LOD for 3-chloro-1,2-propanediol

Component Name	Inj-1		Inj-2		Inj-3		Mean Area	Mean S/N
	Area	S/N	Area	S/N	Area	S/N		
3-chloro-1,2-propanediol	3145	4.1	3099	5.3	3178	3.9	**3141**	**4.4**

A solution containing 1.29 µg/mL of the 3-chloro-1,2-propanediol standard was injected six times. The RSD of areas, deviations of each six replicates from the linear regression curve, and average deviation for each standard were calculated.

Table: 4.0 LOQ for 3-chloro-1,2-propanediol

Component Name	Inj-1	Inj-2	Inj-3	Inj-4	Inj-5	Inj-6	Avg.	%RSD
3-chloro-1,2-propanediol	11611	10658	11994	10258	10018	11457	**10999**	**7.28**

The limit of quantitation and limit of detection values obtained for 3-chloro-1,2-propanediol was within the acceptance criteria.

E. Linearity

The Linearity is determined by injecting the duplicate solutions containing 3-chloro-1,2-propanediol ranging from LOQ to 150% of the specified limit. Perform the regression analysis and determine the correlation coefficient and residual sum of squares. Determine the response factor for 3-chloro-1,2-propanediol impurity with respect to Iohexol. Report the linearity range as the range for determining the impurity. Results obtained are in the table & figure show the line of best fit for peak area versus concentration.

Table: 5.0 Linearity of detector response 3-chloro-1,2-propanediol

Level (%)	Concentration (ppm)	Mean Area
LOQ	1.29	11611
10	2.58	23670
25	6.46	58268
50	12.92	122771
100	25.85	236723
125	32.31	295306
150	38.77	353872
R^2 Value		1.000
% Y-intercept		0.32
Slope		9127.81
Intercept		748.68

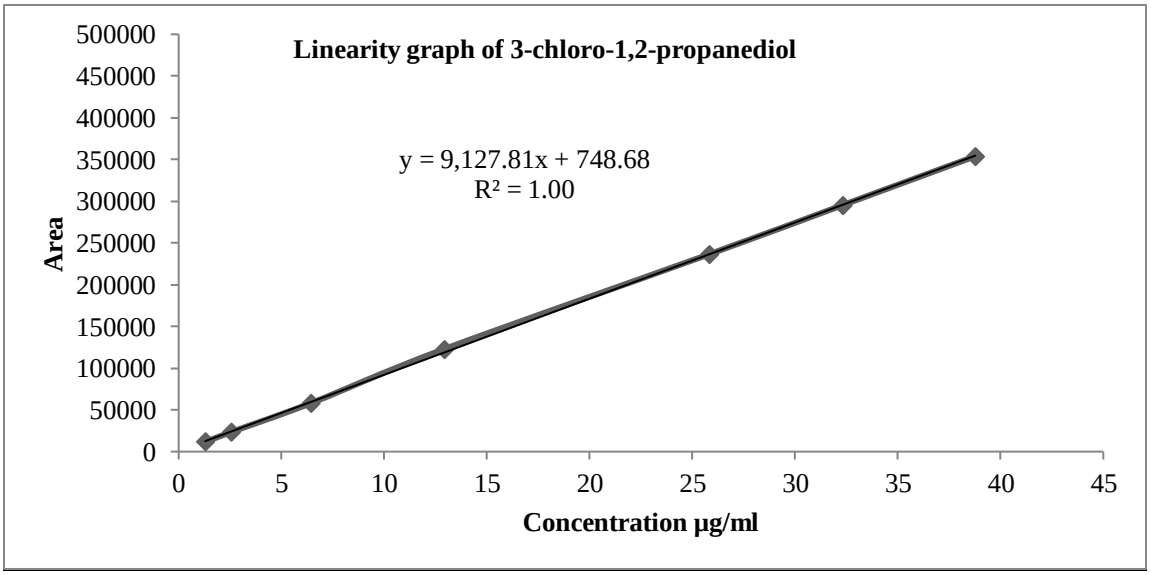

Figure 9.0: linearity of detector response for 3-chloro-1,2-propanediol

F. Accuracy

Recovery of 3-chloro-1,2-propanediol in Iohexol was performed. The sample was taken, and varying amounts of 3-chloro-1,2-propanediol representing LOQ to 150 % of specification level were added to the flasks. The spiked samples were prepared as per the method, and the results are tabulated in **Table 6.0.**

Table 6.0 Recovery results of 3-chloro-1,2-propanediol spiked in Iohexol solution

	3-Chloro-1,2-propanediol				
%Level	**Spiked concentration µg/ml**	**Found concentration µg/ml**	**% Recovery**	**% Mean recovery**	**% RSD**
LOQ	1.29	1.28	99.22	100.00	1.096
	1.29	1.30	100.78		
50	12.92	12.96	100.31	100.89	0.814
	12.92	13.11	101.47		
100	25.85	25.64	99.19	99.30	0.165
	25.85	25.7	99.42		
150	38.77	38.73	99.90	99.75	0.201
	38.77	38.62	99.61		

G. Robustness

To validate the method's robustness, the chromatographic performance at changed conditions was evaluated compared to nominal conditions of the method. The standard solution was injected at a varying flow rate (±0.1 ml/min), pH buffer (±0.1 unit), and column temperature (± 2°C). The results are tabulated in **Table 7.0.**

Table 7.0 Robustness results for 3-chloro-1,2-propanediol

Condition	Investigated value	Peak area	%RSD
pH of mobile phase	4.55	219257	0.773
	4.65	215997	
	4.75	218365	
Flow rate (ml/min)	0.5	218814	0.364
	0.6	217997	
	0.7	219588	
Column temperature (°C)	23	213433	0.617
	25	210997	
	27	211386	

V. Results & Discussion

A simple, economic, accurate, and precise HPLC method was successfully developed. This method was carried out using a Symmetry shield reverse phase C18 (250x4.6mm, 5µm)**.** An injection volume of 30µl is injected and eluted with the mobile phase pH 4.60 phosphate buffer, pumped at a flow rate of 0.6 ml/min. Column temperature 30°C and sample temperature 25°C. A Refractive index (RI) detector was used. The results obtained were accurate and reproducible. The method developed was statistically validated in terms of Selectivity, accuracy, Linearity, precision, robustness, and solution stability.

For Selectivity, the chromatograms were recorded for standard and sample solutions of 3-chloro-1,2-propanediol and Iohexol. Selectivity studies reveal that the peak is well separated from each other. Therefore the method is selective for the determination of 3-chloro-1,2-propanediol in Iohexol. There is no interference of diluent at 3-chloro-1,2-propanediol and Iohexol peaks. The limit of detection (LOD) and limit of quantitation (LOQ) for 3-chloro-1,2-propanediol standard 0.43&1.29µg/mL respectively. The linearity results for 3-chloro-1,2-propanediol in the specified concentration range are satisfactory, with a correlation coefficient greater than 0.99. Calibration curve was plotted, and the correlation co-efficient for 3-chloro-1,2-propanediol was found to be 1.00, respectively. The accuracy studies were shown as % recovery for 3-chloro-1,2-propanediol at the specification level. The limit of % recovered is shown in the range of 90% and 110%, and the results obtained were found to be within limits. Hence the method was found to be accurate. The relative standard deviation values of recoveries obtained for 3-chloro-1,2-propanediol are in the range of 0.17%-1.10%. For Precision studies, six (6) replicate injections were performed. %RSD was determined from the peak areas of 3-chloro-1,2-propanediol found to be 2.66%, respectively. The acceptance limit should be no more than 10, and the results

were within the acceptable limits.

Hence, the chromatographic method developed for 3-chloro-1,2-propanediol in Iohexol is rapid, simple, sensitive, precise, and accurate. Therefore, the proposed method can be successfully applied to routinely analyze the active pharmaceutical ingredients to assure their quality during its formulation.

VI. Conclusion

The proposed RP-HPLC with RI detector method that can quantify impurity 3-chloro-1,2-propanediol in Iohexol at trace level concentration has been developed and validated ICH guidelines. The effectiveness of the method was ensured by specificity, precision, Linearity, and accuracy. Hence, the method well suits for their intended purposes and can be successfully applied for the release testing of 3-chloro-1,2-propanediol into the market.

Acknowledgment

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