Asian Journal of Chemistry; Vol. 29, No. 10 (2017), 2203-2206
ASIAN JOURNAL OF CHEMISTRY
https://doi.org/10.14233/ajchem.2017.20683

Ion Chromatographic Method for Determination of Chloroacetic Acid in Isoproterenol Hydrochloride Drug Substance: A Genotoxic Impurity in Trace Levels

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Received: 3 April 2017; *Accepted*: 30 May 2017; *Published online*: 31 August 2017; AJC-18519

A simple efficient ion chromatographic method developed, optimized and validated for the estimation of trace levels of chloroacetic acid was developed. The validation of this method was realized through specificity, linearity, limit of detection, limit of quantification, precision and accuracy parameters. The analysis was carried out on METROSEP ASUPP 5 (6.1006.530), 250 mm long, 4.0 mm and maintained at ambient conditions.

Keywords: Chloroacetic acid, Isoproterenol hydrochloride, Ion chromatography, Trace levels.

INTRODUCTION

Chlorinated acetic acids draw attention of various synthetic chemists for its use as intermediates in organic synthesis and for its role as substitution of Cl atoms. Among them chloroacetic acid is widely preferred and most significant industrially [1]. In 1841, initially synthesized as chlorination product [2] of acetic acid, it does not occur in nature. Chloroacetic acid in aqueous solution exists as an anion species, where it doesn't have any UV photometric absorption. The estimation of chloroacetic acid was not possible in lower limits in drug substances. But, species like anionic nature in lower limits can be easily estimated using the ion chromatographic technique in conductivity detector mode.

Isoproterenol (isoprenaline) is a sympathomimetic drug (Fig. 1), which imitate the effect of sympathetic nervous system and act solely on β-adrenergic receptors [3]. This drug is listed in 2004 WHO model list as most essential drug, which used in rare disorder, for the treatment of patients suffering with severe bradycardia *i.e.* for short term treatment of heart nodal block [4]. Drug is available as injection containing isoproterenol hydrochloride 20 mcg/mL (1-3 mL). It is recommended as bradyarrhythmias and is administered as an intravenous infusion at dose rate of 1-4 mcg/min related to cardiac disorders.

Chloroacetic acid is used in the synthesis of basic intermediate 3,4-dihydroxy-α-chloroacetophenone of isoproterenol hydrochloride [5]. Fewer literature methods were available in literature for the determination of trace levels of chloroacetic acid in different pharmaceutical drug substances [6-8]. In this communication we report the determination of chloroacetic acid in trace levels and its method validation as per ICH guidelines [9,10].

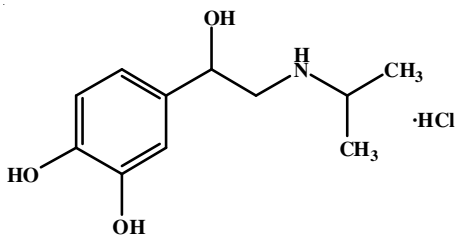

Fig. 1. Structure of isoproterenol hydrochloride

EXPERIMENTAL

Chloroacetic acid used as reference standard was purchased from Sigma-Aldrich. Sodium carbonate, sulfuric acid, sodium-bicarbonate was purchased from E. Merck (Mumbai, india). Water was distilled and purified with Millipore system (Millipore Corporation, India). The known related substances of isoproterenol hydrochloride were prepared at Aurobindo Pharma Ltd. Research Centre, India were use for studies.

Ion chromatography: An ion chromatograph (Metrohm 930 compact IC Flex) with conductometric detector and Metrohm 863 auto sampler or equivalent with Magic IC Net 3.0 or equi-

valent data handling system or An Ion chromatograph (Dionex ICS 5000+) with conductometric detector and AS AP auto sampler or equivalent with Chromeleon 6.8 version or equivalent data handling system, 20 µL loop, Sartorious analytical balance and ultra microbalances.

Mobile phase solution: The mobile phase was a mixture of 4 mM of sodium-bicarbonate and 0.5 mM of sodium carbonate in water. Filter through 0.45 µ finer porosity membrane filter. The analysis was carried out on METROSEP ASUPP 5 (6.1006.530), 250 mm long, 4.0 mm *i.e.*, 5 µm particle diameter column, maintained at ambient conditions. Mobile phase was pumped through the column at a flow rate of 0.5 mL/min. The run time for the standard and sample was 30 min. The injection volume was 20 µL. The retention time of chloroacetic acid is about 18 min. Water is used as diluent. Column oven should be maintained as such that it should not exceed 30 °C.

Suppressor solution: Transfer carefully about 5.6 mL of sulfuric acid to 1000 mL of water. [For Metrohm system] (OR) Transfer carefully about 4 mL of sulfuric acid to 4000 mL of water [For Dionex system].

Standard stock solution: Accurately weigh and transfer about 50 mg of chloroacetic acid reference standard into 100 mL clean dry volumetric flask and add 70 mL of diluent and sonicate to dissolve. Make up to 100 mL with diluent. Further dilute 5 mL of above solution to 100 mL using diluent.

System suitability solution: Weigh about 25 mg of isoproterenol hydrochloride reference sample into a 25 mL clean, dry volumetric flask, add 1 mL of standard stock solution and 15 mL of diluent, sonicate to dissolve and make up to volume with diluent.

Standard solution: Dilute 4 mL of standard stock solution to 100 mL with diluent.

Sample solution: Accurately weigh and transfer about 50 mg of sample into 100 mL clean dry volumetric flask and add 30 mL of diluent sonicate to dissolve. Make up to 100 mL with diluent.

Evaluation system suitability: Inject 20 µL of system suitability solution before and after the analysis into the chromatograph and record the chromatograms. The column efficiency determined from the resolution between chloroacetic acid and chloride is not less than 1.20 min.

Separately inject 20 µL of standard solution, six times into the chromatograph, record the chromatograms and measure the peak areas. RSD for peak areas of six injections of the standard solution is not more than 5 %.

Procedure: Inject 20 µL of diluent, standard solution and sample solution into the chromatograph and record the chromatogram. Examine the diluent chromatogram and no interference peak should be observed at the retention time of chloroacetic acid. Integrate peak due to chloroacetic acid only (retention time of chloroacetic acid is at 18 min).

RESULTS AND DISCUSSION

Method development: Determination of chloroacetic acid in ppm levels, which is strongly retained in the drug using ion chromatography was the main objective of this work. Initial trials were made on instrument with supperssor ion chromatographic mode. Where mobile phase used was 2.2 mM sodium carbonate and 2.8 mM of sodium bicarbonate and column 'Metrosep A Supp 5' with packing material as poly(vinyl alcohol) with quaternary ammonium groups used as stationary phase. As both isoproterenol hydrochloride and chloroacetic acid were soluble in water, solutions were prepared using water as diluents and injected in IC. The retention time of chloroacetic acid standard is about 11.5 min. But, both of the peaks were found to be very close to each other. For the better resolution the method was optimized. In another trial column oven temperature was increased to 35 °C from ambient temperature. But the resolution of standard and sample peaks decreased. Change in temperature has no effect in good resolution.

Better resolution obtained when the concentration of sodium carbonate was decreased to 1.0 mM and bicarbonate increased to 4.0 mM at ambient temperature. Result shown to have better resolution of 1.40 min corresponding to two peaks. Further decreasing sodium carbonate to 0.5 mM, the resolution between both peaks found to have 1.7.

Method validation: In order to determine the chloroacetic acid in isoproterenol hydrochloride drug substance, the above method was validated as per the ICH guidelines. Individually different parameters were analyzed in terms of LOD, LOQ, specificity, linearity, accuracy and precision of sample solution.

Specificity: To verify the selectivity of the method, it was necessary to evaluate the retention times of each impurities present in the drug substances. To identify, each solution was individually prepared as per the methodology. Further the sample solution was organized in a way by spiking the solution with all known related substances of isoproterenol hydrochloride drug substance at about 0.10 % w/w and injected as per the given procedure to conform the number of co-elution peaks from the sample medium. The chromatograms of each analyte clearly shows that the chloroacetic acid peak was well resolved from that of chloride ion present in the isoproterenol hydrochloride drug substance, related substance solution and blank solution, which indicates that the method is selective for determination of chloroacetic acid in isoproterenol hydrochloride. An overlay chromatogram of blank solution, standard solution and sample solution spiked with known amount related impurities of isoproterenol hydrochloride Figs. 2-4.

Limit of detection and limit of quantification: The sensitivity of the method was confirmed by plotting the linearity curve. Different concentration solutions chloroacetic acid was prepared from lower concentration level of 0.1 µg/mL to a higher concentration level of 1.497 µg/mL. The slope (S) and residual standard deviation (SD) were determined from the linearity plot curve. By using a slope (S) and residual standard

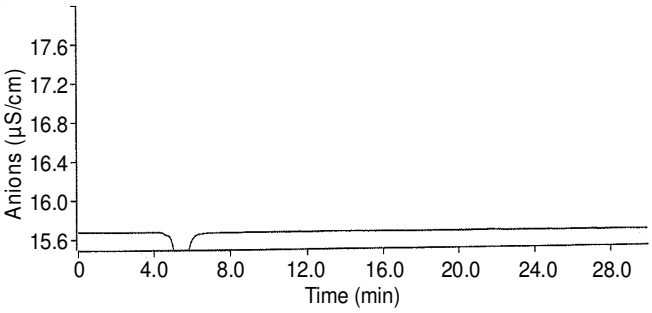

Fig. 2. Chromatogram of blank

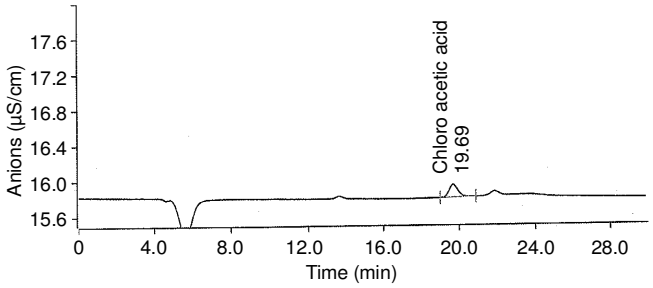

Fig. 3. Chromatogram standard chloroacetic acid

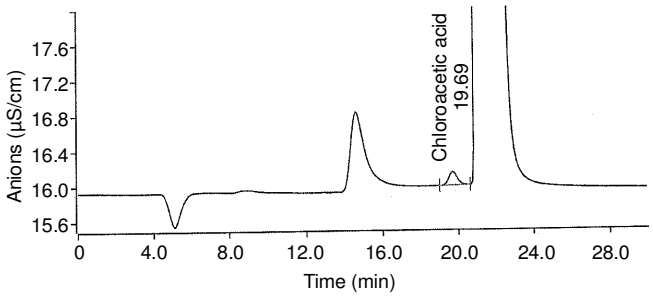

Fig. 4. Chromatogram of isoproterenol hydrochloride drug substance spiked with all known related substances including chloroacetic acid spiked sample

deviation (SD) the limit of quantification and limit of detection of the method was detected, which is being stated in ICH guidelines as one of the three approaches mentioned.

The formulae used for the determination of LOQ and LOD were 10 x STEYX/SLOPE and 3.3 x STEYX/SLOPE, respectively. The predicted LOQ and LOD levels for chloroacetic acid were 0.033 and 0.099 µg/mL, respectively.

To prove the above mentioned levels of LOQ and LOD values different set of solutions were prepared and determined easily in the sample without any ambiguity. The solutions were prepared at the predicted concentration of LOD and LOQ levels and analyzed for six times. The data of six-replicated injection for LOQ and LOD is tabulated in the Table-1.

TABLE-1
PRECISION DATA OF LOD AND LOQ OF CHLOROACETIC ACID

Injection ID	Area for LOD solution (area count)	Area for LOQ solution (area count)
1	0.0026	0.0083
2	0.0031	0.0074
3	0.0022	0.0070
4	0.0027	0.0078
5	0.0026	0.0068
6	0.0027	0.0068
Mean	0.0027	0.0074
RSD (%)	11.1	8.1
Concentration (µg/mL)	0.033	0.099
Concentration (µg/g)	33	99

Linearity: The detector response was established by preparing a series of diluted solutions of chloroacetic acid as per the methodology ranging from 0.099 to 1.497 µg/mL. Each set of solution was injected into the ion chromatographic system and measured the response of chloroacetic acid and concentration of the solution. From the area response of the analyte and concentration of the linear regression line plotted was constructed. From the linear regression line, the correlation coefficient of the regression line and slope was found to be 0.9999 and 0.0754, respectively. The statistical analysis of linear regression line was evaluated and is summarized in Table-2 and linearity plot of concentration of chloroacetic acid *vs.* area response is shown in the Fig. 5.

TABLE-2
LINEARITY DATA SHOWING THE CONCENTRATION OF CHLOROACETIC ACID AND AREA RESPONSE OF EACH CONCENTRATION

S. No.	Concentration (µg/mL)	Area (area counts)
1	0.099	0.0074
2	0.250	0.0191
3	0.499	0.0376
4	0.749	0.0562
5	0.998	0.0747
6	1.248	0.0947
7	1.497	0.1127
Slope		0.0754
Intercept		-0.00001
STEYX		0.0004
Correlation coefficient		0.9999

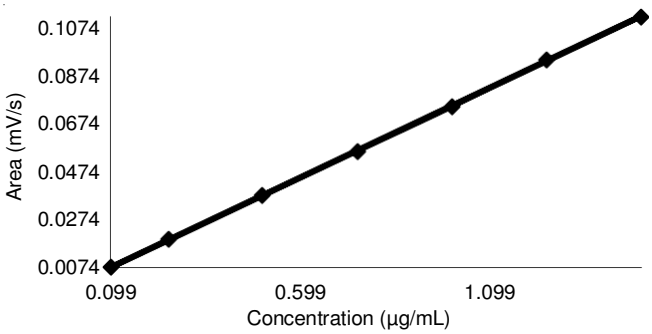

Fig. 5. Linearity plot of concentration of chloroacetic acid *vs.* area response

Accuracy: The recovery or accuracy of the method was tested by adding the chloroacetic acid to the isoproterenol hydrochloride drug substance at three different concentration levels. These concentrations were prepared by adding chloroacetic acid to the isoproterenol hydrochloride about 99, 500, 1000 and 1500 µg/g Sample solutions were prepared in triplicate for each concentration and injected into the IC system and calculate the amount of chloroacetic acid present. The mean recovery was found to be for LOQ level (99 µg/g) is 106.1 % and mean recovery was found to be for 50, 100 and 150 % of specification level (1000 µg/g) is 97.5 %. The results are summarized in Table-3.

Precision: System precision, method precision and intermediate precision were performed using chloroacetic acid standard solution was prepared as per the methodology. In system precision chloroacetic acid solution was injected into the system for six replications and calculated the percentage relative standard deviation of replicate injections (Table-4). In method precision, the sample solution of isoproterenol solution of the same batch substance was prepared in six times as per methodology. The six preparations of the sample solutions were separately injected to the chromatogram and evaluate the repeatability to the test method by calculating the content of the chloroacetic acid in the sample solution for

TABLE-3
RECOVERY DATA OF CHLOROACETIC ACID IN ISOPROTERENOL HYDROCHLORIDE

Concentration/Sample ID	Amount added (µg/g)	Amount recovered (µg/g)	Recovery (%)	Statistical analysis	
LOQ Level Sample-1	98	98	98	Mean	106.1
LOQ Level Sample-2	98	109	109	SD	5.67
LOQ Level Sample-3	99	106	106	RSD (%)	5.30
50 % level Sample-1	493	485	98.4	Mean	98.0
50 % level Sample-2	498	488	98.0	SD	0.40
50 % level Sample-3	497	485	97.6	RSD (%)	0.40
100 % level Sample-1	990	962	97.2	Mean	97.0
100 % level Sample-2	1000	971	97.1	SD	0.21
100 % level Sample-3	1008	976	96.8	RSD (%)	0.20
150 % level Sample-1	1512	1475	97.6	Mean	97.4
150 % level Sample-2	1503	1459	97.1	SD	0.29
150 % level Sample-3	1503	1467	97.6	RSD (%)	0.30
Overall statistical analysis					
		Mean	97.5		
		SD	0.50		
		RSD (%)	0.50		
		95 % Confidence Interval	0.40		

TABLE-4
SYSTEM PRECISION DATA OF
DIFFERENT DAY OF ANALYSIS

Injection	Day-1	Day-2	Day-3	Day-4
Injection-1	0.0746	0.0780	0.0759	0.0756
Injection-2	0.0753	0.0791	0.0769	0.0769
Injection-3	0.0754	0.0791	0.0756	0.0767
Injection-4	0.0751	0.0791	0.077	0.0771
Injection-5	0.0751	0.0788	0.0766	0.0767
Injection-6	0.0752	0.0795	0.0756	0.0771
Mean	0.0751	0.0789	0.0763	0.0767
SD	0.0003	0.0005	0.0006	0.0006
RSD (%)	0.4	0.6	0.8	0.8

the six preparations and the relative standard deviation. The amount of chloroacetic acid and its percentage relative deviation were given in Table-5.

TABLE-5
METHOD PRECISION AND RUGGEDNESS DATA
FOR CHLOROACETIC ACID IN ISOPROTERENOL
HYDROCHLORIDE DRUG SUBSTANCES
AND ITS STATISTICAL DATA

Sample	Chloroacetic acid content (µg/g)	
	Method precision	Intermediate precision
1	983	999
2	1010	986
3	987	976
4	1000	962
5	995	987
6	1008	991
Mean	997	984
SD	10.944	12.911
RSD (%)	1.1	1.3
95 % Confidence interval (CI)	11.487	11.214

Intermediate precision was evaluated by using same lot of isoproterenol hydrochloride sample which was used in method precision and prepared the sample solutions as per the method precision by changing the different lot of the column, system, day, analyst and inject the solution as per methodology. The content of chloroacetic acid in each sample solution was calculated and evaluated the relative standard deviation for the same. The overall relative standard deviation was calculated by clubbing the method precision and intermediate precision data and the results are summarized in Table-5.

Conclusion

A rapid and sensitive ion chromatography method was developed, optimized and validated for the determination of chloroacetic acid. The results of various validation parameters demonstrated that the method is specific, linear, precise and accurate in Isoproterenol hydrochloride drug substance.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the Management of APL Research Centre (A division of Aurobindo Pharma Ltd.) for allowing us to carry out the research work. The authors are also thankful to the colleagues of the Analytical Research Department and Chemical Research Department for their co-operation.

REFERENCES

1. Heidelberg, Beilstein Handbook of Organic Chemistry, Springer, vol. 1, edn 4, pp. 194-197 (1985).
2. G. Koenig, E. Lohmer, N. Rupprich, M. Lison and A. Gnass, Chloroacetic Acids, Ullmann's Encyclopedia of Industrial Chemistry, vol. 2, pp. 473-490 (2012).
3. S. Sweetman, Martindale-The Complete Drug Reference, Pharmaceutical Press, London, edn 34 (2004).
4. R.S.M. Ryan, D.K. Mehta and H.V. Hogerzeil, WHO Model Formulary 2004. World Health Organization (2004).
5. C. Kaiser, D.F. Colella, M.S. Schwartz, E. Garvey and J.R. Wardell, *J. Med. Chem.*, **17**, 49 (1974); https://doi.org/10.1021/jm00247a011.
6. J.M. Sendra and V. Todo, *J. Inst. Brew.*, **96**, 85 (1990); https://doi.org/10.1002/j.2050-0416.1990.tb01019.x.
7. Q. Feng and M. Shifen, *Microchem. J.*, **63**, 317 (1999); https://doi.org/10.1006/mchj.1999.1759.
8. M.G. Pervova, V.E. Kirichenko and K.I. Pashkevich, *J. Anal. Chem.*, **57**, 326 (2002); https://doi.org/10.1023/A:1014902431520.
9. ICH, Q2A Text on Validation of Analytical Procedure, International Conference on Harmonization, October (1994).
10. ICH, Q3B Validation of Analytical Procedure–Methodology, International Conference on Harmonization, November (1996).