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Research Article

DETERMINATION OF TRACEABLE GENOTOXIC IMPURITY CHLOROACETYL CHLORIDE A CARCINOGEN BY LC/MS/MS IN DRUG SUBSTANCES

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ABSTRACT

Objective: A simple liquid chromatography-mass spectroscopy/mass spectroscopy (LC/MS/MS) method was developed for determining chloroacetyl chloride (CAC), which is possible genotoxic impurity at trace levels in pharmaceutical drug substances.

Methods: The suitable LC/MS/MS compatible buffers acetonitrile and ammonium format were used to avoid matrix interferences and for better detectability. This method eliminates the solvent extraction and derivatization steps and requires only the direct injection of the sample into the LC/MS.

Results: The method is specific, accurate, and critical validation parameters have been done and established the limit of quantitation level of 0.003 µg/g.

Conclusion: The method possesses the lowest detection level when compared with other methods referred in this journal.

Keywords: Liquid chromatography-mass spectroscopy, Chloroacetyl chloride, Active pharmaceutical ingredients, Carcinogenic, Drug substance.

INTRODUCTION

Drug substances and their relative compounds such as impurities constitute an important group of genotoxic compounds. Thus, these compounds pose an additive concern to clinical subjects and patients. Genotoxic compounds induce genetic mutations and/or chromosol rearrangements and can, therefore, act as carcinogenic compounds

Chloroacetyl chloride (CAC) is known reagent for acylation and it is used in active pharmaceutical ingredients (API) manufacturing process. It decomposes on heating and producing toxic and corrosive fumes including phosgene and hydrogen chloride. The current international conference on harmonization (ICH) guidelines describes a general concept of qualification of impurities in API (ICH) [1,2]. These guidelines, however, do not clearly stated the acceptable level of impurities in the case of genotoxic impurities. The European medicines agency [3] describes the general framework and practical approaches on how to deal with genotoxic impurities in drug substances. It was stated that, for genotoxic compounds without sufficient evidence for a threshold related mechanism, the guideline proposes a policy of controlling level to "as low as reasonably practicable" principle, where avoiding is not possible. This method is capable of detection of impurities in the lowest possible level.

A simple and rapid capillary zone electrophoretic method [4] for determine carbon tetrachloride. The method has got the detectability of 0.3 µg/g. The sample preparation often involves extractions for enhancement of sensitivity and removal of matrix interferences.

Shimadzu corporation has developed the method for determination of halo acetic acid in tap water by liquid chromatography-mass spectroscopy/mass spectroscopy (LC/MS/MS) method, and detectability was observed to be 0.002 µg/g and same detectability may not be possible as in the case of drug substances due to sample matrix [5].

The another method for determination of 10 halo acetic acid in drinking water by LC/MS/MS and detectability was observed to be 2.73 µg/g which is relatively high [6]. The determining carbon tetrachloride in drinking water and limit of quantitation (LOQ) was 0.1 µg/g [7]. The method for determination of Halo acetic acids by liquid-liquid microextraction. This method has got the LOQ was 0.17 µg/g [8]. The method used for stabilizing reactive GIs and for introducing a detection specific moiety for enhanced detection, i.e., chromophore for UV, also. This method sometimes produces a single compound for several GIs, and thus it becomes non-specific. The technical report by Gwendolyn G. Howard from US army biomedical research [9] has developed the method for analysis of halogenated acetic acid by IC but detectability was 1.93 µg/g.

Ion chromatograph method for determination of CAC in carboceisteine was developed and LOQ was achieved only 0.017 µg/g [10], but in general IC technique takes normally more time for stabilization and need to be carried out in highly inert conditions to avoid ionic interferences. High-performance LC-UV/MS (HPLC-UV/MS) method is widely applied technique for determining genotoxic impurities in trace level owing to their inherent high sensitivity and precision [11-13].

The novelty of the research work is primarily aimed to achieve the lowest possible detection level of the impurity which is achieved by LC/MS/MS with optimization of the mass parameters. This method shall be applied to any API.

METHODS

Method development

The analytical method development for the estimation of CAC content in drug substance by LC/MS.

Equipment, materials and reagents

LC/MS (LC-Shimadzu MS-API4000) with analyst software, HPLC components Binary pump, Degasser, Autosampler, Thermo-stated column compartment, Analytical balance, Column Atlantis HILIC 250 mm × ID 4.6 mm, 5 m. Water (Ultra-pure), acetonitrile (HPLC grade 99.9%), ammonium formate (AR grade 99.8%), CAC.

Instrument condition

The detection of the CAC was done by reverse phase liquid chromatography with a mass spectrometer. 10 mM ammonium formate buffer and acetonitrile were used as mobile phase, Stationary phase is Atlantis HILIC 250 mm × 4.6 mm, 5 m, the flow rate of mobile phase is 0.4 mL/minute, injection volume 10.0 L, column thermostat temperature 25°C, ion source ESI, negative ion source (API 4000), m/z 92.8/34.9, temperature 500°C, ion spray voltage - 4500, the mixture of water and acetonitrile used as diluent.

RESULTS AND DISCUSSIONS

Atlantis HILIC Silica columns were used in combination with high organic mobile phases to provide retention of analyte that are simply too polar to be retained by reverse phase chromatography. HILIC separation mechanism provides orthogonal analyte selectivity compared to traditional chromatographic reversed-phase approaches. The ammonium formate buffer which is LC/MS compatible and has got good PH. working range (2.2-4.7) and support filling gap at low PH. The method was taken for complete analytical method validation as per ICH guidelines [14] and USP monograph [15].

Specificity and system suitability

To verify that the analytical system is working properly and can give accurate and precise results, CAC standard solution (0.66618 µg/ml) was injected into LC/MS/MS. It is observed from the tabulated data, the % relative standard deviation (RSD) for the 6 replicate injections was 4.97 % (Table 1). It is calculated by using equations:

$$\%RSD=\frac{\text{Standard deviation }(SD)}{\text{Mean}}\times 100$$

Standard deviation:

$$S=\sqrt{\frac{\sum(x-\bar{x})^2}{n-1}}$$

x: Individual value, x (bar): Mean of the distribution, n: Number of observations.

It can be concluded that the system suitability parameter meets the requirement of method validation. The chromatograms of blank and CAC pertains to system suitability found satisfactory (Figs. 1 and 2).

Linearity

The linearity of an analytical method is its ability to elicit test results that are directly or by a well-defined mathematical transformation, proportional to the concentration of an analyte in samples within a given range. To determine the linearity of chloroacetic acid, 5.15 mg of chloroacetic acid standard substance diluted to 0.1236 µg/g and further the linearity solutions prepared (Table 2) from 150% (0.0092 µg/g) to 5% (0.00031 µg/g) as different concentration level and Injected each level of linearity solutions. Plotted a graph of concentration of the solution in mg/mL (X-axis) against area response (Y-axis). Calculated the regression coefficient between concentration and area response.

The equation of a straight line is y = mx + b.

From the Table 2, it is clear that the area response of chloroacetic acid is linear from lower (5%) level to higher level (150%) of the specified concentration. The correlation coefficient and regression coefficient is greater than 0.995 (Fig. 3).

Limit of detection/LOQ

LOQ is the lowest amount of analyte in a sample that can be quantitated with acceptable precision (Table 3) under the stated experimental conditions. It is calculated using equation,

$$\text{LOQ}=\frac{(10\times\text{residual SD})}{\text{Slope}}$$

Table 1: System suitability for standard CAC (n=6 runs six replicates)

System suitability	Specificity compound name	m/z
Run 1=3022.485	CAC	92.8/34.9
Run 2=3383.897	Chloroacetic acid	92.8/34.9
Run 3=3149.482		
Run 4=3348.411		
Run 5=3009.483		
Run 6=3166.604		
Mean x=3180.0603		
SD[a]=158.07217		
RSD[b]=4.97		

[a]Standard deviation, [b]Relative standard deviation, CAC: Chloroacetyl chloride

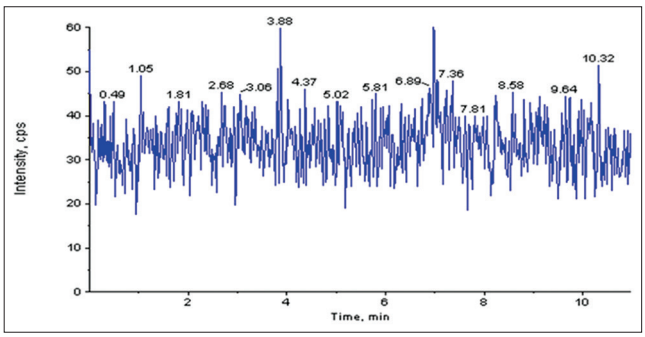

Fig. 1: Blank chromatogram

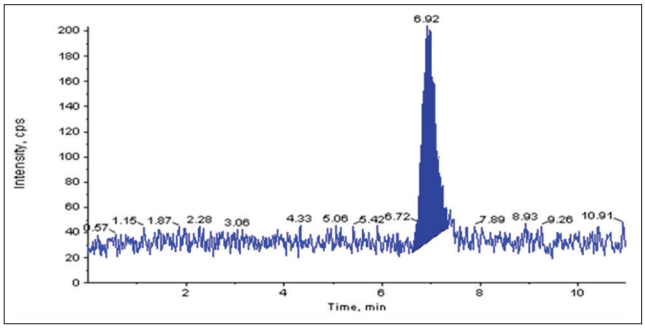

Fig. 2: Chloroacetyl chloride chromatogram

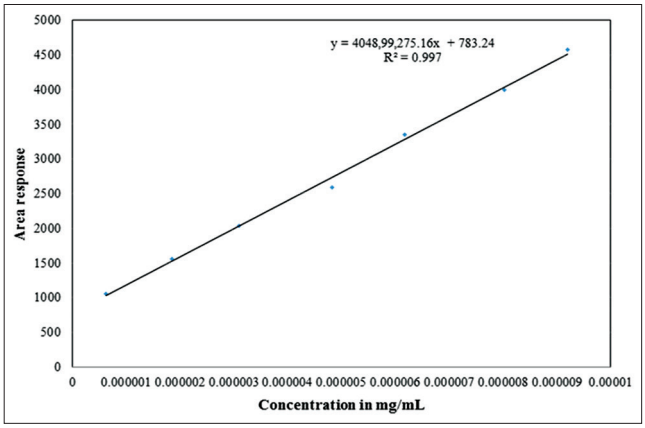

Fig. 3: Linearity from limit of quantification level to 150% of chloroacetyl chloride

Table 2: Linearity

Linearity level	CAC in (mg/ml)	Area response
5	0.0000003	0.000
10	0.0000006	1060.4780
30	0.0000019	1562.7300
50	0.0000031	2039.8050
80	0.0000049	2596.3200
100	0.0000062	3346.4160
130	0.0000080	3991.0330
150	0.0000092	4573.3280
Regression coefficient		0.997
Slope		404899275.16
Intercept		783.24

CAC: Chloroacetyl chloride

Table 3: LOQ precision LOQ solution 0.3 µg/g

CAC	Analyte area
Run 1	1607.418
Run 2	1774.493
Run 3	1752.839
Run 4	1828.623
Run 5	1567.515
Run 6	1789.093
Mean	1719.9968
SD	106.34573
RSD	6.18

LOQ: Limit of quantification, CAC: Chloroacetyl chloride, RSD: Relative standard deviation, SD: Standard deviation

Table 4: Precision

Precision	80%	100%	130%
Run-1	2390.022	3010.441	3600.522
Run-2	2395.047	3103.015	3521.038
Run-3	2301.859	3210.569	3590.458
Run-4	2299.101	3205.201	3499.985
Run-5	2458.581	3193.101	3501.259
Run-6	2401.014	3100.012	3520.189
Average	2374.271	3137.057	3538.909
SD	62.29	79.69	44.85
% RSD	2.623	2.540	1.267
µg/g	0.0047	0.0061	0.0079

RSD: Relative standard deviation, SD: Standard deviation

Table 5: Method precision established with different level

Method precision			
% level	Spiked µg/g	Mean area	% RSD
80	0.0047	4671.804	1.98
100	0.0061	5432.687	1.48
130	0.0079	6497.333	3.49

RSD: Relative standard deviation

Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of a homogeneous sample. The precision of the analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of series of measurements. The system precision is checked by using standard CAC substance to ensure that the analytical system is working properly.

The stock solution was prepared containing CAC. Made a series of dilutions such that the diluted solutions will have a concentration, which is equivalent to 80%, 100%, and 130% with respect to 0.3 µg/g of specification limit for CAC.

In precision 6 times specified concentration (0.0061 µg/g) and 130% (0.0079 µg/g) and 80% (0.0047 µg/g) level CAC standard was injected and calculated the % RSD (Table 4).

Method precision and accuracy

In method precision, a homogenous sample of a single batch analysed. This indicated that the method has given consistent results for a single batch drug substance.

Pre-analyzed drug substance was spiked with CAC at 80%, 100% and 130% of the specification limit (considering 0.3 µg/g of specification limit for CAC). The spiked drug substance was analysed and calculated the RSD for observed (Table 5). CAC in six replicate trials. Established the % recovery of chloroacetyl chloride to individual trial. To determine the method precision calculated the % RSD for six replicated injection (Table 6).

Drug substance with the above-validated method, drug substance were analysed for the estimation of CAC content. Prepared the drug substance 20.0 mg/mL (Table 7) for three different batches and checked the results LC/MS/MS.

CONCLUSION

A specific and accurate estimation with critical validation parameters for above method for determining the traceable level of CAC was described in this article. The validation results met with guidelines requirement. The sample preparation and determination is very simple, as this method is direct determination and there is no derivatization. The method was applied to the drug substances to demonstrate the

Table 7: CAC estimation in drug substance

Drug sample	Amount weighed	Area response	Calculated Conc. of CAC in sample (in µg/g)
Trial-1	200.44	2026.860	0.0030
Trial-2	200.21	1038.424	0.0003
Trial-3	200.31	1275.677	0.0010

CAC: Chloroacetyl chloride

Table 6: Method accuracy and % recovery

Accuracy	Drug substance in mg			% Recovery		
Trial	For 80% level	For 100% level	For 130% level	80% level	100% level	130% level
Trial-1	200.22	200.59	200.09	104.02	102.53	103.61
Trial-2	199.89	202.58	200.50	102.65	103.64	104.32
Trial-3	198.25	202.69	201.50	102.36	103.80	104.74
Trial-4	202.55	201.89	201.93	104.21	100.17	103.61
Trial-5	199.02	202.63	197.69	100.66	101.91	99.33
Trial-6	198.59	203.08	209.50	99.91	103.78	108.62
Mean				102.30	102.64	104.04
SD				1.74	1.43	2.97
% RSD				1.70	1.40	2.85

RSD: Relative standard deviation, SD: Standard deviation

suitability of the method and observed that the results were found satisfactory.

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