

Original Article

DEVELOPMENT AND EVALUATION OF CLASS-1 SOLVENT 1, 2-DICHLOROETHANE AND DIMETHYL SULPHATE AS A GENOTOXIC IMPURITY IN SILDENAFIL CITRATE DRUG SUBSTANCE BY USING GC-MS TECHNIQUE

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ABSTRACT

**Objective:** Method for determination of 1, 2-Dichloroethane (DCE) and Dimethyl sulphate (DMS) was required in Sildenafil citrate. 1, 2-Dichloroethane is class-1 solvent and Dimethyl sulphate is genotoxic impurity so sensitive, highly efficient and selective analytical technique has been developed by gas chromatography-mass spectrometer.

**Methods:** The best results were achieved by using DB-624 30 m x 0.25 mm x 1.4 µm column with electron impact ionization technique quadruple mass analyzer. Gas chromatography column DB-624 is an intermediate polarity column with 6 % cyanopropyl phenol, 94 % dimethyl polysiloxane as stationary phase.

**Results:** Limit of detection (LOD) and Limit of quantification (LOQ) for 1, 2-Dichloroethane are 0.5 µg/g and 1.5 µg/g respectively (0.02 µg/mL and 0.06 µg/mL). LOD and LOQ values obtained for Dimethyl sulphate are 1.6 µg/g and 5.0 µg/g respectively (0.06 µg/mL and 0.20 µg/mL). Also method is evaluated for accuracy at 50 to 150 % of the evaluation limit. Results obtained for accuracy are very well within the acceptable criteria. Calibration curve showed good linearity over the concentration range LOQ to 150 % of the evaluation level. The correlation coefficient values observed are 0.99416 and 0.99141 for 1, 2-Dichloroethane and Dimethyl sulphate which is very good at such a low level.

**Conclusion:** Based on the validation data it is concluded that the method is sensitive, precise, accurate and linear as per the parameters conducted for the validation activity and is suitable for the routine use.

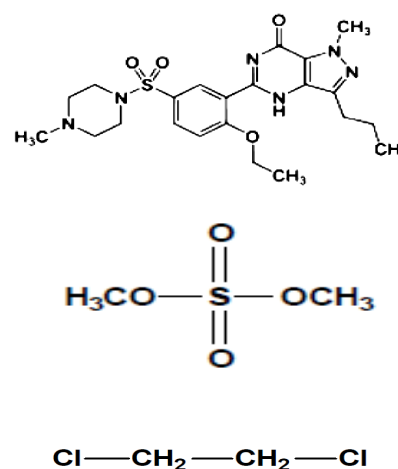
**Keywords:** Development, Validation, Sildenafil Citrate, 1, 2-Dichloroethane, Dimethyl sulphate, Gas chromatography-Mass spectrometer

INTRODUCTION

Sildenafil is a drug used to treat erectile dysfunction and pulmonary arterial hypertension (PAH). Manufacturing process of Sildenafil Citrate involves the use of Methanol, Acetone and Dichloromethane as process solvents. Dichloromethane contains 1, 2-Dichloroethane as one of the related impurity, which is also a Class I solvent with limit of 5 µg/g and also Dimethyl sulphate is also one of the possible impurities during the manufacturing process of Sildenafil citrate. So the study is proposed and conducted for the method development and further validation of method for determination of 1, 2-Dichloroethane and Dimethyl sulphate in Sildenafil citrate drug substances. Recommended maximum daily dose for Sildenafil citrate is 100 mg. Based on the genotoxic impurity guideline, the limit for the Dimethyl sulphate is decided as 15 µg/g.

The results obtained after completion of validation are all within the set acceptance criteria. During the manufacturing process of Sildenafil citrate process solvents used are Methanol, Acetone and Dichloromethane. Dichloromethane does contain 1, 2-Dichloroethane as potential impurity. Also, due to use of Methyl chloro sulphate and methanol in the process, analytical evaluation of Dimethyl sulphate in Sildenafil citrate was required. As per the IARC monograph of 1, 2-Dichloroethane, is a potential carcinogen and is listed in 2B group (Possibly carcinogenic to humans) [1]. Also as per the International conference on harmonisation guideline for residual solvents, 1, 2-Dichloroethane is a Class 1 solvent with Limit 5 ppm [2]. As per the IARC monograph of Dimethyl sulphate, it is listed in 2A group (Probably carcinogenic to humans) and also it is genotoxic as per the final evaluation [3]. Various approaches are utilized while developing method for the content of Dimethyl sulphate like extraction [4] as well as derivatisation [5]. Based on daily dose limit of Dimethyl sulphate is proposed and evaluated at 15 µg/g level [6] and as per ICH guideline limit of 1, 2-

Dichloroethane is 5 µg/g. The analytical tools used during these analytical approaches are GC-MS as well as LC-MS [7-8] as limit of evaluation is very low. Various methods in the literatures involve determination of sildenafil by HPLC [9-22], by UV spectrophotometry [23] and by HPTLC [24]. In the present work we have developed a new, simple precise method for determination of 1, 2-Dichloroethane and dimethyl sulphate in sildenafil citrate by gas chromatography-mass spectrometer.



Sildenafil, Dimethyl Sulphate 1, 2-Dichloroethane

Fig. 1: Chemical structure of Sildenafil and impurities proposed for analysis

## MATERIALS AND METHODS

### Materials

All the reagents used during the development and validation activity are of the analytical grade. Three commercial batches of Sildenafil citrate are used during the validation and evaluation activity. Diluent used for the activity is Dichloromethane and is used for preparation of standards and sample preparations and is procured from Rankem. Also, 1, 2-Dichloroethane and Dimethyl sulphate with certified purity are procured from Thomas Baker and Rankem respectively.

### GC-MS operating conditions

Instrument used for the Method development and validation activity is Shimadzu GCMS QP-2010 with Quadruple mass analyzer and software control used is GCMS solution version 2.61. DB-624 column, length 30 meter, internal diameter 0.25 mm, film thickness 1.4  $\mu\text{m}$  is used for performing validation and evaluation. Column oven programme used is as initial temperature 50  $^{\circ}\text{C}$  and initial holding time of 5 min, then the temperature is raised to 220  $^{\circ}\text{C}$  at the rate 20  $^{\circ}\text{C}/\text{minute}$  and the final temperature hold is kept for 2 minutes. Injector port temperature is maintained at 220  $^{\circ}\text{C}$ . Ion source temperature and Interface temperature are programmed at 200  $^{\circ}\text{C}$  and 220  $^{\circ}\text{C}$  respectively. Helium is used as the carrier gas with constant linear velocity 36.3 cm/sec. SIM mode is used for the analysis. Based on Mass spectra of both the components m/z values screened for SIM mode are 62 and 95 for 1, 2-Dichloroethane and Dimethyl sulphate respectively.

### Preparation of solutions for analysis

Dichloromethane is used as blank and is also used as diluent for the preparation of standards as well as sample solutions.

For standard solution preparation, 0.05 g of 1, 2-Dichloroethane and 0.15 g of Dimethyl sulphate is diluted to 100 mL with diluent and then 1.0 mL of the resulting solution is diluted to 50 mL. Further 1.0 mL of the resulting solution is diluted to 50 mL and is used as final standard solution. Final standard solution concentration is 5 and 15  $\mu\text{g}/\text{g}$  (0.2 and 0.6  $\mu\text{g}/\text{mL}$  with respect to sample) respectively for 1, 2-Dichloroethane and Dimethyl sulphate.

Sildenafil Citrate sample solutions were prepared by weighing and diluting 0.200 g of sample to 5 mL with Dichloromethane sonicated the resulting solution for 5 minutes and supernatant solution is used for the analysis.

Method used for estimation of Limit of detection (LOD) and Limit of Quantification (LOQ) is Slope intercept method. Prediction solutions were prepared in the range 0.3 to 3.8  $\mu\text{g}/\text{g}$  (0.012 to 0.152  $\mu\text{g}/\text{mL}$  with respect to sample) for 1, 2-Dichloroethane and 0.7 to 11.2  $\mu\text{g}/\text{g}$  (0.03 to 0.45  $\mu\text{g}/\text{mL}$  with respect to sample).

Linearity solutions were prepared in the range 1.5 to 7.5  $\mu\text{g}/\text{g}$  (0.06 to 0.30  $\mu\text{g}/\text{mL}$  with respect to sample) for 1, 2-Dichloroethane and 5.0 to 22.5  $\mu\text{g}/\text{g}$  (0.2 to 0.9  $\mu\text{g}/\text{mL}$  with respect to sample) using appropriate dilutions which is from LOQ to 150 % of the evaluation limit.

## RESULTS AND DISCUSSION

### Method development

To start with method development activity different diluents are considered based on solubility of analytes and recovery of those in Sildenafil citrate. Dichloromethane is used as diluent after using different diluents like methanol, Ethanol, N, N-Dimethyl formamide etc. Columns used for the development trials are DB-5, DB-1, DB-624 and Rtx-1301 with different dimensions. Finally based on peak performance and minimum baseline interference, DB-624 (Stationary phase: 6% cyanopropylphenyl) column with length 30 meter, internal diameter 0.25 mm and 1.4  $\mu\text{m}$  film thickness is chosen as suitable.

Solvent cut time is also proposed for the analysis as it will reduce the unnecessary exposure of MS to diluent components. Solvent cut time is kept up to 5.0 minutes from the start of acquisition. Also, MS acquisition is kept 'ON' between 6.5 to 7.5 minutes and 11.5 to 11.2

minutes, this is the range in which 1, 2-Dichloroethane and Dimethyl sulphate gets eluted out of the column. Purpose behind proposing the MS acquisition range is to avoid the unnecessary exposure of MS to sample components which are not of our analysis interest.

Further based on the full scan of 1, 2-Dichloroethane and Dimethyl sulphate in the m/z range 10-500 amu; SIM mode m/z is finalized. For 1, 2-Dichloroethane, m/z is kept as 62 while for Dimethyl sulphate; m/z is kept as 95. Spectra of the analytes are compared with reference spectra in the NIST library.

### Method validation

Method is developed, proposed and completed for the content of 1, 2-Dichloroethane and Dimethyl sulphate in Sildenafil citrate. Validation activity is planned on the basis of International Conference on Harmonisation validation guideline. While starting every activity, six injections of standard solution were injected in the system as system suitability solution. System suitability criteria kept is as 15 % relative standard deviation.

### Specificity, Limit of detection and limit of quantification

Specificity is performed by injecting all the solvents in the process and concluded that no interference is observed at the retention time of analytes peaks.

Calibration curve method is used for establishment of LOD and LOQ. For LOD and LOQ prediction standard solutions ranging from 6 % to 75 % of the evaluation limit concentration are injected. Further based on the calibration curve and resulting Slope and STEYX values LOD and LOQ values are obtained. LOD and LOQ values obtained for 1, 2-Dichloroethane are 0.5  $\mu\text{g}/\text{g}$  and 1.5  $\mu\text{g}/\text{g}$  respectively (0.02  $\mu\text{g}/\text{mL}$  and 0.06  $\mu\text{g}/\text{mL}$ ). LOD and LOQ values obtained for Dimethyl sulphate are 1.6  $\mu\text{g}/\text{g}$  and 5.0  $\mu\text{g}/\text{g}$  respectively (0.06  $\mu\text{g}/\text{mL}$  and 0.20  $\mu\text{g}/\text{mL}$ ). Details of the LOQ and LOD values are as shown in the table 1 and 2 respectively.

### Linearity

Linearity of the method is proved on complete quantification range i.e. from LOQ to 150 % of the Specified limit. Linearity curve is plotted with peak areas against the concentration of respective analyte. Linearity equation observed for 1, 2-Dichloroethane is ( $y = 812.7x + 250.6$ ) and for Dimethyl sulphate is ( $y = 610.1x - 272.0$ ). The correlation coefficient values observed are 0.99416 and 0.99141 for 1, 2-Dichloroethane and Dimethyl sulphate which is very good at such a low level.

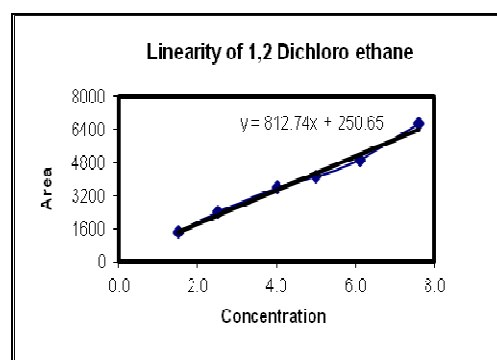


Fig. 2: Linearity graph of 1, 2-Dichloroethane

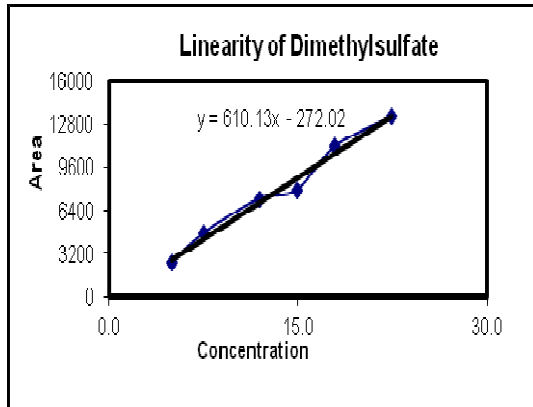
### Precision and Accuracy

Method precision activity is completed by spiking the Sildenafil citrate sample with standard solution at the specification limit. System precision activity is performed by injecting six standard solutions and % relative standard deviation observed is 0.7 and 1.2 respectively for 1, 2-Dichloroethane and Dimethyl sulphate. The mean results obtained at three accuracy levels i.e. 50 %, 100 % and 150 % are in the range of 92 % to 99 % for 1, 2-Dichloroethane and

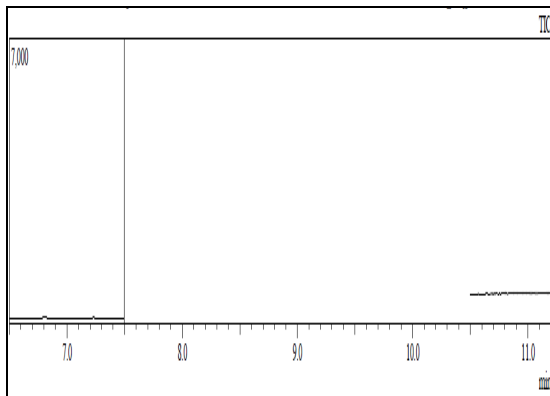
Dimethyl sulphate. All the results of the three levels are observed well within the acceptance range of 80-120 %.

**Mass spectral analysis**

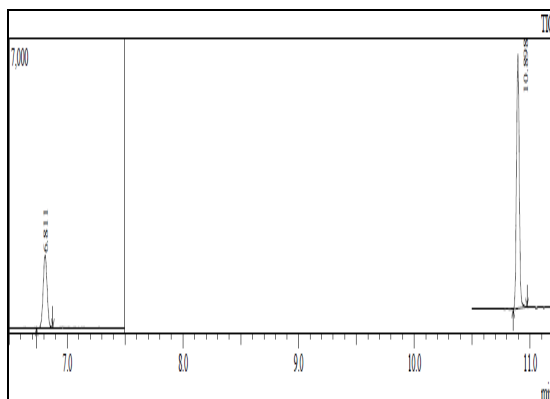
As per the elution order and scan chromatogram retention time range of 1, 2-Dichloroethane is 6.5 to 7.5 minutes and that of Dimethyl sulphate is 10.5 to 11.2 minutes. The Mass spectrum of 1, 2-Dichloroethane and Dimethyl sulphate is as shown in figures. Major fragments observed in mass spectrum of 1, 2-Dichloroethane are 98, 62, 49 and 27. Major fragments observed in mass spectrum of Dimethyl sulphate are 125, 95, 79, 66, 45 and 31. The spectrum of both the analytes match to the reference spectrum.



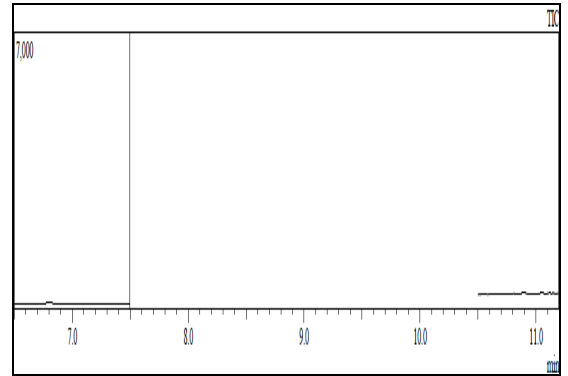
**Fig. 3: Linearity graph of Dimethyl sulphate**



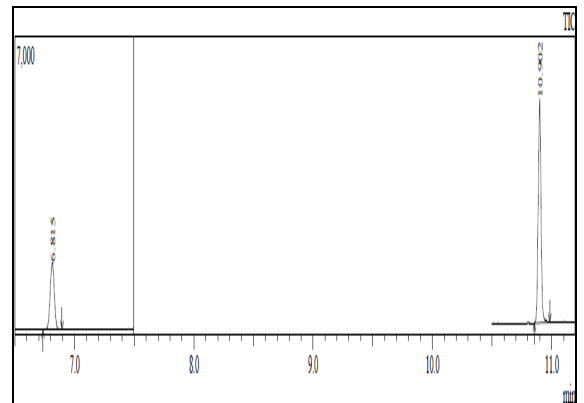
**Fig. 4: Chromatogram of Blank solution**



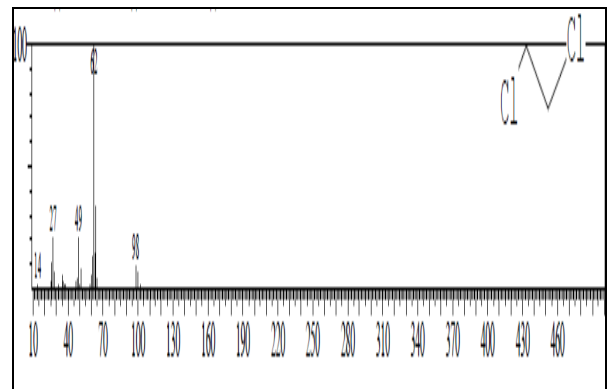
**Fig. 5: Chromatogram of Standard solution at Specification concentration SIM mode (Retention time: 6.811 min: 1, 2-DCE, 10.898 min: DMS)**



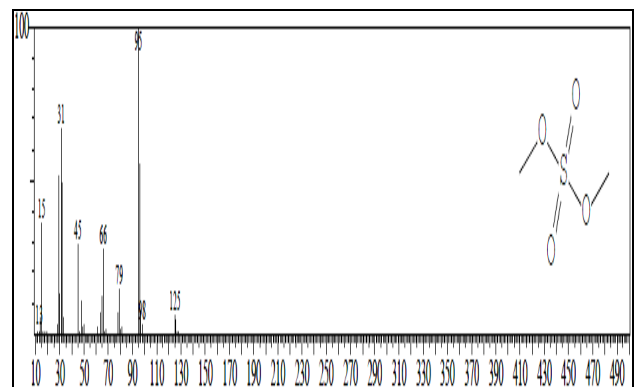
**Fig. 6: Chromatogram of Sample solution**



**Fig. 7: Chromatogram of Spiked sample solution (Retention time: 6.815 min: 1, 2-DCE, 10.902 min: DMS)**



**Fig. 8: Mass spectrum of 1, 2-Dichloroethane**



**Fig. 9: Mass spectrum of Dimethyl sulphate**

**CONCLUSION**

Method is developed and validated for the content of 1, 2-Dichloroethane and Dimethyl sulphate in Sildenafil citrate. Method is highly precise, Accurate, Linear, sensitive for the analysis purpose. Based on the validation parameters performed and further analysis of three commercial batches of Sildenafil citrate, method is suitable for use in routine analysis.

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