QUANTIFYING TWO GENOTOXIC IMPURITIES IN DARUNAVIR UTILISING GC/MS SINGLE QUADRUPOLE ANALYZER USING ELECTRON IONISATION APPLICATION

Mohinish Sahai¹, and Nayakanti Devanna²

¹Department of Chemistry, Jawaharlal Nehru Technological University, Ananthapuramu-515002, (Andhra Pradesh) India
²Director, Oil Technological & Pharmaceutical Research Institute, Jawaharlal Nehru Technological University, Ananthapuramu-515002, (Andhra Pradesh) India

Corresponding Author: mohinish_sahai@rediffmail.com

ABSTRACT

This document presents a previously unreported GC/MS approach for the design and validation of the contemporaneous estimation for prospective genomic toxic impurities, Carbon tetrachloride (CTC) and 1,2-Dichloroethane (DCE), in Darunavir (DAR). The measure limits of this approach ranged from 1.0 to 6.1 parts/million for CTC and 1.3 to 7.4 parts/million for DCE. This approach has demonstrated excellent linearity (coefficient of correlation squared value more than equal to 0.999), preciseness of method (0.6%, N=6) for CTC and (1.0%, N=6) for DCE, and system suitability (1.7%, N = 6) for CTC and (1.3%, N = 6) for DCE in accordance with relative standard deviation in percent. Recoveries obtained at the lowest specification and higher level of spiked DAR samples ranged between 96% and 98%. This GC/MS approach has shown its suitability and functionality in assessing CTC and DCE simultaneously in DAR 's regular quality determinations.

Keywords: Impurities, Genotoxic, Darunavir, Carbon tetrachloride, 1,2-Dichloroethane, Gas Chromatography, Mass Spectrometer.

INTRODUCTION

Solvents of Organic nature are routinely and widely utilized in pharmaceutical substances and formulation products. Varied manufacturing practices are in place to remove these hazardous solvents during intermediate or final stages, but complete removal of some solvents is a tedious and complex task that requires highly advanced instrumentation and techniques. Pharmacopoeia’s have proposed a limit of control for most of the widely used synthesis solvents and classified them into different classes. Carbon tetrachloride (CTC) and 1,2 dichloroethane (DCE) are classified as class-1 solvents with a limit, 4ppm and 5ppm, respectively and are considered toxic and hazardous for human intake. Carbon tetrachloride is a hepatotoxin of high potency known to cause liver damage by way of its biologically mediated transformation into metabolites of toxic nature.

International Agency for Research on Cancer (IARC) has kept CCl₄ in group 2B, citing it as a possible carcinogen in humans. ¹, 2-Dichloroethane also, as per its IARC monograph, is a potent carcinogen and hence listed in group 2B. ² Most class-1 solvents are considered toxic and potential carcinogens, hence accurate identification and quantification at or below its limit of control are extremely necessary during quality control of pharmaceutical dosage forms for human use. Long-time use and accumulation of these hazardous residual solvents may impact human health to a large extent.

The practice of using organic solvents like carbon tetrachloride and 1,2 dichloroethane at any stage of synthesis or purification of an active pharmaceutical constituent leads to the probability of their residuals amounts in final drug substances/products. The allowable quantity of any impurity in ppm is the proportion of toxicological risk threshold value (µg per day) and maximum allowable dose of pharmaceutically active ingredient (gm per day).³

Rasayarn J. Chem., 14(4), 2754-2760(2021)
http://dx.doi.org/10.31788/RJC.2021.1446444

This work is licensed under a CC BY 4.0 license.
Darunavir is an orally administered HIV-1 protease inhibitor (PI), nonpeptidic in nature, that is used synergistically with ritonavir, as a part of therapy in HIV-1 infections.\(^6\)

The permitted intake of CTC and DCE in DAR is calculated based on permitted daily exposure\(^7\) and comes to 4 ppm and 5 ppm per day, respectively. This value was used to establish the specification limits for the concentrations of CTC and DCE in the current method evaluation.

Monitoring and controlling the impurity-related quality parameter of a drug substance or drug product is as valuable as evaluating its efficacy.\(^8\) Determination of Residual solvents in pharmaceutical products were also attempted by GC/HS and GC/MS utilizing SPME technique.\(^9\) Literature also shows an assay method for the simultaneous quantification of some newly introduced antiretroviral agents: like Raltegravir, maraviroc, darunavir, and etravirine using LCMS.\(^10\)

LC–MS/MS has previously been used for the structural characterization of stress degradation products in Darunavir and also for developing stability-indicating assay method for Darunavir.\(^11\) Fast and simultaneous determination of Darunavir and eleven other antiretroviral drugs has been previously attempted by a liquid chromatography technique which was coupled with electrospray ionization tandem mass spectrometry.\(^12\)

Development and evaluation of genotoxic solvent impurities like 1, 2-dichloroethane and dimethyl sulphate have been attempted using GC-MS technique in sildenafil citrate drug substance.\(^13\)

Thermal desorption-GC/MS has previously been used to determine residual solvents in pharmaceuticals.\(^14\) Clayton B’Hymer has presented an excellent review of the use of Gas chromatographic and alternative techniques for residual solvent techniques.\(^15\) Rami Reddy et al have also developed an HPLC method for determining Darunavir Ethanolate that is stability-indicating.\(^16\) The presence of CTC and DCE contaminants in DAR is not reported to date by any author according to our best of literature survey. The current document delineates the initial approach on GC/MS for the design and validation for contemporaneous determination of CTC and DCE contaminants in DAR.

**EXPERIMENTAL**

**Instrumentation and Approach Details**

CTC and DCE analysis and determination were executed on a gas chromatographic system (Agilent, model-7890B) accompanied by a mass spectrometer (Agilent, model-5977A). Segregation of CTC and DCE was obtained on a ZB-624 column of 60 m in length, the internal diameter of 0.32 mm and 1.8-micron thickness of the film and a gaseous flow rate of 2 ml/min. The oven temperature scheme started at 40°C at initial, held for time 5 mins and then ramped up at the rate of 8°C per min till 160°C, held for 0 min and then further ramped up at the rate of 30°C per/min till 280°C, and the end temperature hold for 5 mins. The automated Injector assembly temperature was modulated at 180 °C. The prepared sample was introduced in the Headspace sampler in split type 4:1. Quad and Source temperatures were modulated at 150 °C and 250 °C individually. The discovery of CTC and DCE was achieved using mass spectrometric assembly in selected (SIM) mode with an evaluation of (ions – 117 m/z demonstrating CTC and 62 m/z demonstrating DCE). The data capture and processing were evaluated using Mass Hunter software (Agilent).

**Chemicals**

Reference samples of CTC, DCE and DAR with the potency of 99.9%, 99.9% and 99.5%, respectively, were obtained in the form of gifted samples from Sun Bio (Bangalore, India). Dimethylsulphoxide (DMS) sourced through Merck India was utilized as a solvent for dilution.

**CTC and DCE Solution**

Stock CTC and DCE solution in DMS were prepared at a concentration of 500 ppm. Working CTC and DCE solutions were subsequently prepared by diluting the stock with DMS to attain a concentration of 5 ppm. Six calibrating dilutions of CTC and DCE at varied levels (CTC – 1.0 to 6.1 in ppm and DCE – 1.3 to 7.5 in ppm) were prepared from the stock by appropriately diluting with the DMS diluent.

**DAR Solution as Sample**

DAR solution at a concentration of 20000 ppm was prepared by dissolving the drug substance appropriately in DMS diluent.
Methodology for Estimation of CTC and DCE in DAR Sample Solution

The column was conditioned at 280ºC for approaching about 30 min. The gas chromatography system was equilibrated at 40ºC to obtain a steady baseline minimum for 30 min. Subsequently, the following solutions were injected into the system for analysis: - DMS solvent blank (N=1), CTC and DAR solutions of standard (N=6) and DAR solution of the sample (N=1). The peak areas of CTC and DCE were obtained by employing the settings described in the section “Instrumentation and approach details.” The following formula gave the concentration of the impurities -CTC and DCE in ppm – present in the DAR solution of the sample:

\[
\text{Conc. of Impurity (ppm)} = \frac{[(A_{\text{IT}}) - (A_{\text{BL}})]}{(A_{\text{STD}}) - (A_{\text{BL}})} \times \frac{(W_{\text{STD}})}{(D_{\text{STD}})} \times \frac{(D_{\text{TEST}})}{(W_{\text{TEST}})} \times P \times 10^6
\]

As per equation, \(A_{\text{BL}}\) = Area of contaminant in DMS diluent solvent chromatogram; \(A_{\text{IT}}\) = Area of contaminant CTC and DCE in Test solution chromatogram; \(A_{\text{STD}}\) = Mean area of CTC and DCE in standard solutions; \(W_{\text{STD}}\) = Amount in weights of CTC and DCE in solution of standard; \(W_{\text{TEST}}\) = Amount in weight of DAR in solution of sample; \(D_{\text{STD}}\) = Factor of dilution for CTC and DCE solution of standard; \(D_{\text{TEST}}\) = Factor of dilution for DAR solution of sample; \(P\) = CTC and DCE potency.

RESULTS AND DISCUSSION

Method Optimization

Feasibility studies were done with gas chromatography to enhance the resolution and response, along with a reduction in the interference for CTC and DCE estimation. Different columns having various chemistry were tried, inclusive of DB-5MS (30 m in length, internal diameter of 0.32 mm and 0.25-micron thickness of film), ZB-624 (30 m in length, internal diameter of 0.32 mm and 1.8-micron thickness of film )and ZB-624 (60 m in length, internal diameter of 0.32 mm and 1.8-micron thickness of film ). GC/MS settings like, oven temperature schedule and rate of gas flow were also assessed and optimized. Repercussions of keeping pressure in constant mode and keeping the flow in the constant mode were also was evaluated. Trials on ZB-624 (60 m in length, internal diameter of 0.32 mm and 1.8-micron thickness of film ) column with optimum oven settings and Gaseous flow rate (check section “Instrumentation and approach details”) were found showing more appropriate results as compared to the other investigated trials. The discovery of CTC and DCE was performed in the selected ionization mode of evaluating (SIM) (ions – 117 m/z demonstrating CTC, and 62 m/z demonstrating DCE, see Fig.-1).
Validation
System Suitability
The suitability of the system was evaluated using the blank (diluent) and a repeat of six injections of CTC and DCE standard solutions. The method was considered suitable because the relative standard deviation in responses of peak areas from six injections for CTC (1.7%) and DCE (1.3%) was lower than 15%.17-19

Specificity
The method-specific nature was evaluated using the blank (diluent), CTC and DCE solution of standard, DAR solution of the sample without spiked and DAR solution of sample spiked with CTC and DCE. The amount of CTC and DCE in standard and spiked DAR solutions was about 4 ppm for CTC and 5 ppm for DCE. The representative chromatographs of these 4 samples are presented in Fig.-2. It can be seen from the Fig.-2, that there is no observable interferences observed at retention time of CTC and DCE in chromatograms of blank (Diluent) and DAR sample solution. The retention time of CTC and DCE was found to be 12.3min and 12.6 min, respectively, in chromatogram with DAR spiked with CTC and DCE impurities and in chromatogram with CTC and DCE solution of standard.

Fig. -2: Chromatograms [a] DMS Blank (Diluent) [b] Working CTC and DCE Solution [c] DAR Solution of Sample [d] DAR with CTC and DCE spiked

Limits for Detection (LD) and Quantitation (LQ)
LD and LQ were confirmed based on the S/N ratio evaluation at a known concentration. LD was assessed at 0.1 ppm and 0.4 ppm for CTC and DCE, respectively and LQ was assessed at 1 ppm and 1.3 ppm for CTC and DCE, respectively. The relative standard deviation for CTC and DCE area response (Table-1) was calculated for 6 injections at the respective concentrations (Table-1) and was found to be less than 15% indicating the precision of LQ values to perform analysis.

| Solvent | 1   | 2   | 3   | 4   | 5    | 6    | %RSD |
|---------|-----|-----|-----|-----|------|------|------|
|         | LQ  |     |     |     |      |      |      |
| CTC     | 6856| 6945| 6765| 6812| 6884 | 6988 | 1.2  |
| DCE     | 1550| 1643| 1544| 1623| 1565 | 1587 | 2.5  |
|         | LD  |     |     |     |      |      |      |
| CTC     | 1330| 1412| 1353| 1387| 1402 | 1398 | 2.3  |
| DCE     | 267 | 300 | 287 | 265 | 311  | 298  | 6.5  |
Linearity
Linearity of the method was evaluated by determining six concentrations that ranged from the above-established LQ concentration to 150% of the specification concentration limit of the impurities (4 ppm for CTC and 5 ppm for DCE). To be specific, the concentrations ranged from 1.0 to 6.1 ppm for CTC and 1.3 to 7.5 ppm for DCE. To prove the linearity of the method, graphs of CTC and DCE were plotted between areas and the respective level concentrations (Fig.-3). Correlation coefficient in squared form (correlation of coefficient, squared residual sum, slope and Intercept at Y axis) values for CTC and DCE were calculated and are presented in Table-2. The Square of correlation coefficient value for CTC and DCE was higher than value 0.999, depicting acceptable linearity in the selected range of concentrations for the impurities.

Table-2: Demonstrating Linearity of CTC and DCE

| Parameter                        | CTC          | DCE          |
|----------------------------------|--------------|--------------|
| Range of Linearity               | 1.0 to 6.1 ppm | 1.3 to 7.5 ppm |
| Correlation of Coefficient       | 0.9999       | 0.9999       |
| Square of the Correlation Coefficient | 0.999       | 0.999       |
| Slope                            | 6523.4       | 1249.8       |
| Residual Sum of Square           | 133686.8     | 6026.83      |
| Y-Intercept                      | 556.06       | 168.81       |

Fig.-3: CTC and DCE Linearity Graphs

Precision
The precision of the system was evaluated by examining one blank (diluent) injection and six injections of the CTC and DCE solution of standard. Calculated relative standard deviation in response peak areas for CTC (1.7%), and DCE (1.3%) was lower than 15%, establishing acceptable precision of the system to evaluate CTC and DCE using this method.

The method’s precision was evaluated by analyzing six injections of the DAR solution (20000 ppm) spike done with CTC and DCE at a specified limit value. The mean value and relative standard deviation of CTC and DCE are calculated (Table-3). The relative standard deviation of the six response peak areas was lesser than 15%, suggesting acceptable repeatability in quantifying CTC and DCE concurrently in this method.
Table-3: Demonstrating Method Precision for CTC and DCE

| Solvent | The Amount Estimated (ppm) of Sample | Mean ppm | RSD |
|---------|-------------------------------------|----------|-----|
|         | 1        | 2        | 3     | 4     | 5     | 6     |       |
| CTC     | 4.02     | 3.98     | 4.03  | 3.98  | 4.00  | 3.98  | 4.00  | 0.6          |
| DCE     | 4.87     | 4.94     | 4.81  | 4.87  | 4.81  | 4.86  | 4.86  | 1.0          |

Accuracy

The accurateness of the method was established by studying three samples: -the DAR solution (20000 ppm) spiked with CTC and DCE at LQ (CTC – 1.0 ppm and DCE – 1.3 ppm), spiked with CTC and DCE at 100% (CTC – 4.0 ppm, and DCE – 5.0 ppm) and spiked with CTC and DCE at 150% (CTC – 6.0 ppm and DCE – 7.4 ppm) of specified limit value. The recovery at each of the three levels is presented in Table-4. The percent mean value of CTC and DCE from 3 levels was within limits of 80 to 120% (Table-4), demonstrating acceptable accuracy of this method in determining CTC and DCE simultaneously in the method.

Table-4: Demonstrating Accuracy for CTC and DCE

| Solvent | Mean Percent Recovered* |
|---------|-------------------------|
|         | LQ limit | 100% limit | 150% limit | Mean |
| CTC     | 96.4     | 98.1       | 98.1       | 97.5  |
| DCE     | 96.2     | 98.0       | 98.1       | 97.4  |

* Mean for three recovery values for LQ and 150% and six recoveries for level-100%

CONCLUSION

The GC/MS method has been developed and validated in this present study to determine CTC and DCE impurities in DAR simultaneously. Based on the observed results, the method has been specific, precise, sensitive, linear, and shows acceptable accuracy. Hence, this GC/MS method can be used to control the mentioned genotoxic impurities and evaluate the quality of the drug substance DAR.

ACKNOWLEDGEMENT

The authors wish to thank Mr. Pavan (Sun Bio ltd.) for the gift samples and solvents along with the provision of adequate infrastructure required to conduct this research.

REFERENCES

1. USP 43-NF 38 (467) Residual Solvents, 6712.
2. V. L. Kubie, M. W. Anders, Chemico-Biological Interactions, 34, 201(1981), https://doi.org/10.1016/0009-2797(81)90131-9
3. IRIS (2010) Toxicological review of carbon tetrachloride (Final report). U.S. Environmental Protection Agency (EPA), In Support of Summary Information on the Integrated Risk Information System (IRIS), Washington, DC, EPA/635/R-08/005F, 2010.
4. IARC Monographs: Program on the Evaluation of Carcinogenic Risk to Humans for 1, 2-Dichloroethane, 71(7), 501(1999).
5. R. Kroes, J. Kleiner, A. Renwick, Toxicological Sciences, 86, 226(2006), https://doi.org/10.1093/toxsci/kfi169
6. K. McKeage, C. M. Perry and S. J. Keam, Drugs, 69, 477(2009), https://doi.org/10.2165/00003495-200969040-00007
7. Guideline on Setting Health-based Exposure Limits For Use in Risk Identification in The Manufacture of Different Medicinal Products in Shared Facilities, EMA/CHMP/ CVMP/ SWP/169430/2012.
8. L. Müller, R.J. Mauthe, C.M. Riley, Regulatory Toxicology and Pharmacology, 44, 198(2006), https://doi.org/10.1016/j.yrtph.2005.12.001
9. Costin C. Camarasu, Ma’ria Mezei-Szuts, Ga’bor Berto’k Varga, Journal of Pharmaceutical and Biomedical Analysis, 18, 623(1998), https://doi.org/10.1016/s0731-7085(98)00276-3
10. A. Fayet, A. Béguin, B. Zanolari, S. Cruchon, N. Guignon, A. Telenti, M. Cavassini, H. F. Günthard, T. Buclin, J. Biollaz, B. Rochat, L. A. Decosterd, Journal of Chromatography B, 877(11-12), 1057(2009), https://doi.org/10.1016/j.jchromb.2009.02.057
11. R. Nageswara Rao, B. Ramachandra, B. Sravan, Sara Khalid, *Journal of Pharmaceutical and Biomedical Analysis*, 89, 28(2014), https://doi.org/10.1016/j.jpba.2013.10.007
12. Rob ter Heine, Carolien G. Alderden-Los, Hilde Rosing, Michel J. X. Hillebrand, Eric C. M. van Gorp, Alwin D. R. Huitema, Jos H. Beijnen, *Rapid Communications in Mass Spectrometry*, 21, 2505(2007), https://doi.org/10.1002/rcm.3119
13. Nalavade Atul Kakasaheb, K. Ramakrishna, V. Srinivasrao, *International Journal of Pharmacy and Pharmaceutical Sciences*, 6(4), 2014.
14. K. Hashimoto, K. Urakami, Y. Fujiwara, S. Terada, C. Watanabe, *Analytical Sciences*, 17(5), 645(2001), https://doi.org/10.2116/analsci.17.645
15. Clayton B’Hymer, *Pharmaceutical Research*, 20, 337(2003), https://doi.org/10.1023/A:1022693516409
16. B.V. Rami Reddy, G. Jyothi, B.S. Reddy, N.V.V.S.S. Raman, K. Subhash Chander Reddy, C. Rambabu, *Journal of Chromatographic Science*, 51(5), 471(2013), https://doi.org/10.1093/chromsci/bms165
17. Mohinish Sahai, Nayakanti Devanna and Rahul Kumar Rajput, *Rasayan Journal of Chemistry*, 14(2), 1081(2021), https://doi.org/10.31788/RJC.2021.1426208
18. USPNF 2021 (1467), Issue 2, 3.
19. Le Dinh vu, Phan tan Lap and Le Van Tan, *Rasayan Journal of Chemistry*, 11(4), 1537(2018), https://doi.org/10.31788/RJC.2018.1144061 [RJC-6444/2021]