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Estimation of Benzyl Cyanide as a Genotoxic impurity in Primidone API and its pharmaceutical dosage forms by using GC-FID

Uppalapati Vidyamani, Department of Chemistry, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India.

P. Bharat, Department of Chemistry, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India.

P.V. Surendra Guptha, Department of Chemistry, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India.

Dittakavi Ramachandran, Department of Chemistry, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India.

S.Sambamurthy Raju, Department of Chemistry, KVR, KVR & MKR College, Khajipalem, Guntur, Andhra Pradesh, India.

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Abstract: To develop a Sensitive, accurate, precise and linear GC-FID method for quantitate estimation of Benzyl Cyanide as an impurity in Primidone API at ppm level and validated as per ICH guidelines. This GC-FID method was developed and validated for the trace level analysis of an impurity by using Column: VF-624ms, 30m*0.32mm ID*1.8µm. Carrier Gas: Nitrogen. Flow: 1.0 mL/min. Injector temperature: 200°C. Detector temperature: 250°C. Split ratio: 10. GC Oven program: Initial temperature 100°C hold for 2.0 min and then increase the temperature up to 200°C at Ramp rate 10°C/min hold for 10.0 min and then increase the temperature up to 240°C at Ramp rate 20°C/min hold for 36.0 min. The method was linear for Benzyl Cyanide as an impurity in Primidone 2.5 ppm to 15 ppm respectively. The coefficient of correlation (r) not less than 0.999. The limit of detection and limit of quantification obtained were 0.76 ppm and 2.5 ppm with respect to Sample concentration. The method was fully validated, complying FDA and ICH guidelines and obtained results were within acceptance criteria. The method was successfully validated to determination and quantification of Benzyl Cyanide impurity in Primidone API. Hence, the method holds good for the routine trace analysis of Benzyl Cyanide impurity in Primidone API and its pharmaceutical dosage forms in pharmaceutical industries.

Keywords: Benzyl Cyanide, Primidone, GC-FID, Method Development, Method Validation

1. Introduction:

In keeping with modern regulatory recommendations, it's miles vital that the genotoxic impurities doubtlessly harm the DNA at very low-level vulnerability. Genotoxic materials are the chemical compounds that harm an organism via adverse its genetic fabric. There are three primary effects that Genotoxins will have on organisms by way of affecting their genetic facts. Genotoxins may be cancer causing agents or mutagens or teratogens. Potential genotoxic impurities maximum probable rise up in the course of synthesis, purification, and storage need to be diagnosed. As in keeping with USFDA pointers concerning the bounds of genotoxic impurities, a most of 1.5 µg in keeping with a day is the exposure restriction **[1]**. Benzyl cyanide **(figure-1)** can also present in the API of Primidone. A method primarily based on GC-FID is feasible within limits of time, ease of application, sensitivity, and cost. Notwithstanding the importance of the difficulty, no technique is up to now said for the simultaneous dedication of these impurities in API of Primidone. Primidone **(figure-2)** is an anticonvulsant drug Chemically Primidone is known as 5-ethyl-5-phenyldihydropyrimidine-4,6 (1H,5H)-dione **[2]**.

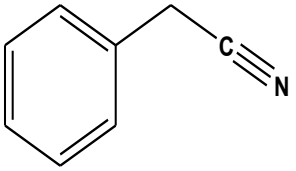

Figure 1: Structure of Benzyl cyanide

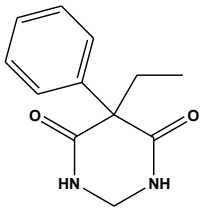

Figure 2: Structure of Primidone

The goal of the modern-day studies paper is to quantify Benzyl cyanide as potential genotoxic impurity in Primidone API and its pharmaceutical dosage forms at trace level. During tremendous literature search for no analytical strategies for the determination of Benzyl cyanide impurity in Primidone API by using GC-FID. There had been also HPLC pharmacopeial methods published for Primidone content in its pharmaceutical dosage forms.

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Numerous courses are available for the determination of Primidone content in pharmaceutical dosage paperwork by way of HPLC **[3-8].** There was a publication on analytical methods for the dedication of different genotoxic and nitrosamines impurities in different drugs through GC-MS/MS and LC-MS/MS **[9-15].** But, there is no approach posted till date for the trace level quantification of Benzyl cyanide impurity in Primidone API and its pharmaceutical dosage forms by using GC-FID.

2. Materials and Methods

Chemicals and reagents

Dimethyl sulfoxide (DMSO) and Benzyl Cyanide were purchased from Sigma-Aldrich. Primidone API is taken from a famous neighborhood studies Laboratory.

Instruments and Chromatographic conditions

Chromatography was performed on Shimadzu chromatographic system equipped with a Shimadzu GC-2010 system with FID. Data acquisition and integration were performed using GC solution software. The instrument parameters described below were set up to estimation of Benzyl cyanide impurity. The GC method conditions are, Column: VF-624ms, 30m*0.32mm ID*1.8µm. Carrier Gas: Nitrogen. Flow: 1.0 mL/min. Injector temperature: 200°C. Detector temperature: 250°C. Split ratio: 10. GC Oven program: Initial temperature 100°C hold for 2.0 min and then increase the temperature up to 200°C at Ramp rate 10°C/min hold for 10.0 min and then increase the temperature up to 240°C at Ramp rate 20°C/min hold for 36.0 min. Total Run time: 60.0 min. Injection Volume: 0.5µL.

3. Preparation of Solutions

Diluent

Used Dimethyl sulfoxide.

Preparation of Benzyl Cyanide Standard Stock Solution (250 ppm)

Weighed about 75 mg of Benzyl Cyanide and transferred into 100 mL volumetric flask. Add 70 mL diluent, sonicate for about 2 minutes to dissolve and dilute to the volume with diluent. Pipetted out 5.0 mL of this solution in 50 mL volumetric flask and dilute to the volume with diluent.

Preparation of Standard Solution (10 ppm)

Take and transfer about 2.0 mL above standard Stock solution into 50 mL volumetric flask and makeup to the volume with diluent. (This 10 ppm standard solution has been prepared to Primidone Concentration).

Preparation of Primidone Sample Solution (300 mg/mL)

Weighed and transferred accurately about 3.0g of the sample into a 10 mL volumetric flask. Add 8 mL diluent, sonicate for about 2 minutes to dissolve and dilute to the volume with diluent.

Preparation of Primidone tablet (300 mg/mL)

Twenty tablet pills had been weighed and overwhelmed to a first-rate powder. The powder equal of 300 mg Primidone become taken in a 100 mL volumetric flask containing diluent and kept sonication for 10 min and made up to mark with diluent. The resultant mixture becomes filtered through 0.45 µm nylon filter. The desired concentration for the drug was obtained through correct dilution, and the analysis was observed up as within the widespread analytical manner.

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Calculation

The Nitrosamine Impurities content was calculated from,

$$ppm = \frac{\text{Impurity area in Sample}}{\text{Impurity area in BC Standard solution}} \times \frac{\text{BC Standard Solution Concentration}}{\text{Sample Solution Concentration}} \times 10^6$$

4. Chromatographic Method Development

The GC approach has been developed based totally on Gas chromatographic conditions. The column screening has been performed for higher top resolution among Benzyl cyanide and diluent peak. After choosing the column, fine-tune the technique to exchange the Injection, Detector temperatures, and Oven program. The oven program tuning is useful to lessen the total run time with proper resolution. The GC column approach optimization details are shown in **Table-1**.

Table 1: GC-Method optimization details

Name of the column	USP Resolution	USP Tailing factor	USP Plate count
ZB-1 (30 m × 0.53 mm, 5.0 µ)	above 2.0	about 2.15	Plate count is good (Around 10000)
ZB-624 (30 m × 0.53 mm, 3.0 µ)	above 3.0	about 1.85	Plate count is good (Around 20000)
VF-624ms (30 m × 0.32 mm, 1.8 µ)	above 5.0	about 1.05	Plate count is good (Around 35000)
ZB-Wax (30 m × 0.32 mm, 1.8 µ)	above 1.5	about 1.75	Plate count is good (Around 10000)

5. Method Validation

All of the Parameters of validation conducted using ICH recommendations **[16].**

Specificity

The ICH guidelines define specificity as the capacity to evaluate unequivocally the analyte within the presence of additives that may be expected to be present, which includes impurities, degradation merchandise, and matrix additives. Analytical techniques becomes tested for specificity to measure as it should be quantitative Benzyl cyanide in drug Primidone drug substances. The benzyl cyanide eluted at 13.79 min. Plate count and tailing factor should be with the ICH criteria. The typical zoomed chromatograms shown in **Figure 3.**

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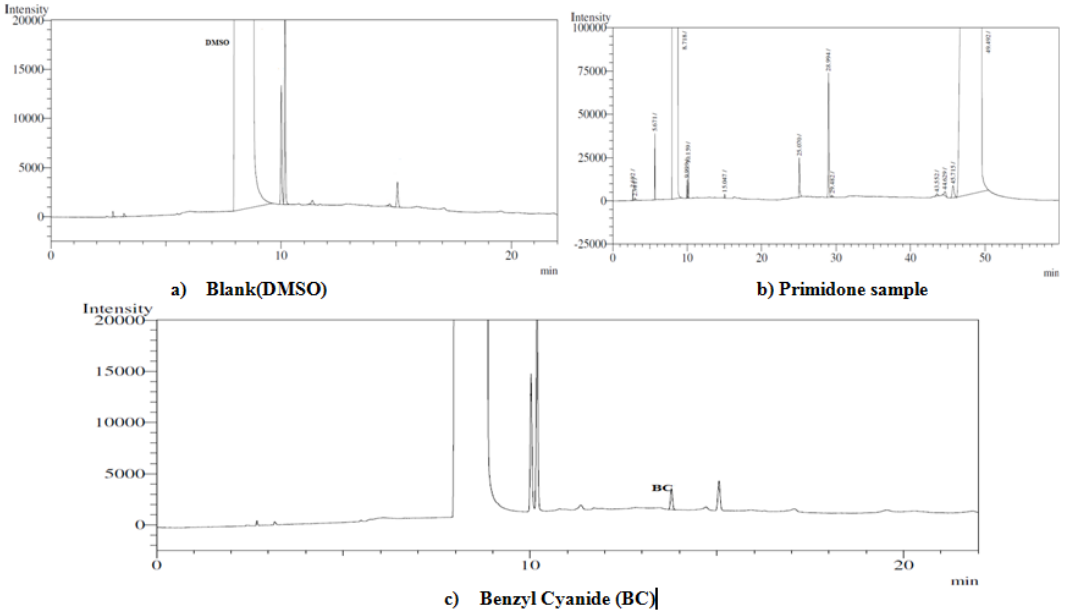

Figure 3: a) Blank (DMSO); b) Primidone sample; c) Benzyl cyanide

System and Method Precision

Obtained six replicate injections and six replicate preparations of BC standard solution (10ppm) as per GC-FID method conditions. Recorded the peak RT and areas and calculated % RSD. The obtained %RSD should be not more than 10%. The %RSD for the BC standard solution is below 7.0%, which is within the limits hence method is precise. The data is shown in **Table 2.**

System precision		
Average (n=6)	13.78	5695
STDV (n=6)	0.004	384
% RSD (n=6)	0.03	6.75
Method precision		
Average (n=6)	13.79	4985
STDV (n=6)	0.002	269
% RSD (n=6)	0.01	5.39

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ were established by the using of signal-to-noise ratio(s/n). As per United States pharmacopeia (USP) S/N ratio for LOD should be greater than or equal to 3.0 and for LOQ should be greater than or equal to 10. The data and zoomed chromatograms of LOD and LOQ are presented in **Table 3 & Figure 4.**

Table 3: LOD and LOQ data

Name of Impurity	LOD Con.(ppm)	LOQ Con.(ppm)	LOD Area(n=3)	LOQ Area(n=6)
Benzyl cyanide	0.76	2.50	770	1619

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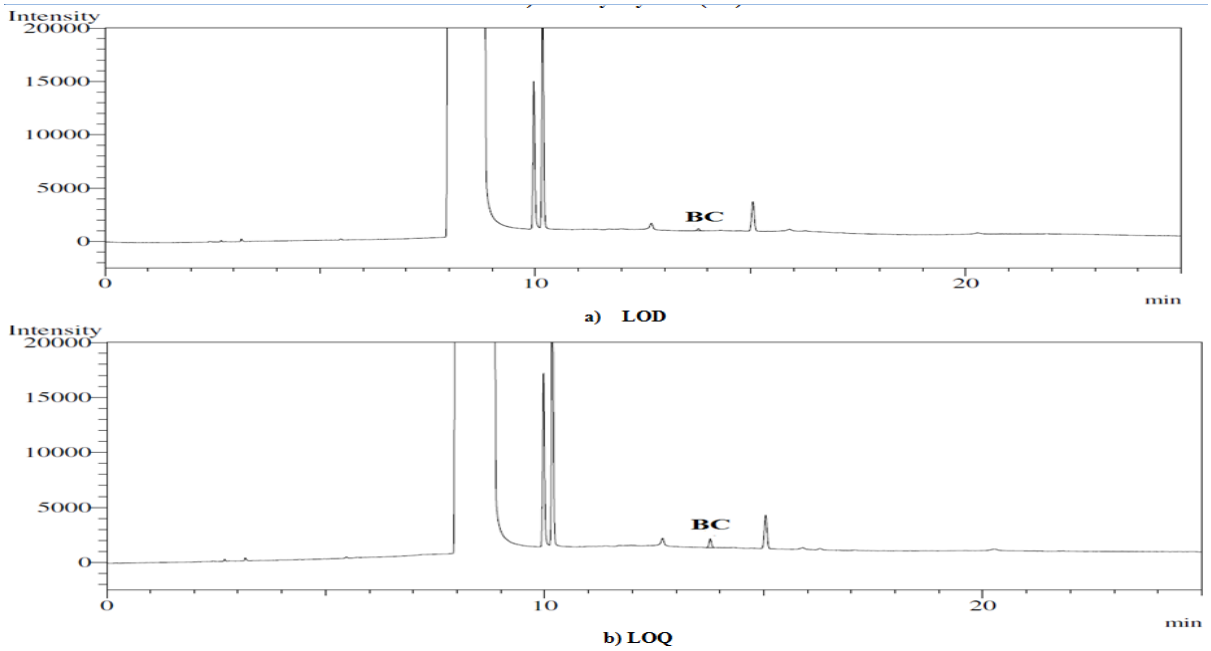

Figure 4: a) LOD; b) LOQ

LOQ-Precision

The Benzyl cyanide was prepared at LOQ level (2.5 ppm) absolute and injected in six replicates. The average area was obtained for Benzyl cyanide is 1619.The %RSD for Benzyl cyanide peak area response of Standard six injections should not more than 10.0% and retention time is not more than 1.0%. The precision data and typical chromatograms at LOQ concertation as shown in **Table 4.**

Table 4: LOQ Precision data for Benzyl cyanide

No. of Injections (n=6)	Benzyl cyanide	
	RT (min)	Area
1	13.79	1689
2	13.78	1622
3	13.78	1489
4	13.78	1698
5	13.78	1589
6	13.78	1625
ACVG	13.78	1619
STDV	0.005	76
% RSD	0.03	4.70

Linearity & Range

The linearity of the method was determined by making injections of Standard Benzyl cyanide impurity over the range 2.5, 5.0, 7.5, 10.0, 12.5 and 15.0 ppm. Two replicates were performed at each level. The calibration curves were obtained with the average of peak area ratios of two replicates. The correlation coefficient (r2) value for Benzyl cyanide was found to be higher than 0.99 and the calibration curves were linear within the range. These results revealed an excellent linearity. The linearity data for the Benzyl cyanide as shown in **Table 5** and typical Linearity Calibration curve is shown in **Figure 5.**

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Table 5: Linearity data

Con.(ppm)	Benzyl cyanide
2.5	1577
5	2860
7.5	4515
10	5594
12.5	7082
15	8582
r2	**0.999**

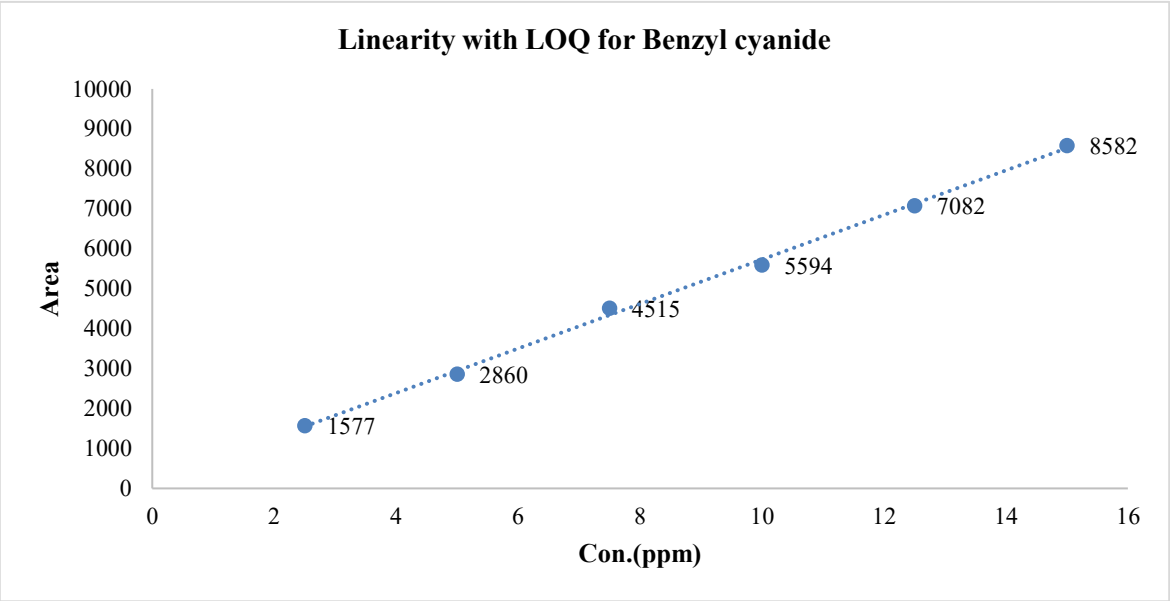

Figure 5: Correlation graph

Accuracy

A known amount of sample was spiked with Benzyl Cyanide standard solutions with three different levels LOQ 2.5 ppm (LOQ%), 5.0 ppm, 10.0 ppm and 15.0 ppm in triplicates preparations. From accuracy data, the % recovery of Benzyl Cyanide was found within the limits (100 ±15%). Results indicates that the method has an acceptable level of accuracy. The accuracy data and typical chromatograms are presented below **Table 6.**

Table 6: Accuracy data for Benzyl Cyanide

Benzyl cyanide Area					
No. of Preparations	**Primidone Sample**	**Sample+5 ppm (n=3)**	**Sample+10 ppm (n=3)**	**Sample+15 ppm (n=3)**	**Sample+2.5 ppm (n=3)**
Prep-1	0	3126	5591	8146	1552
Prep-2	0	3006	5740	8066	1425
Prep-3	0	3136	5669	8249	1611
Average	0	3089	5667	8154	1529
STD Benzyl cyanide Average area		5695			
%Recovery		108.5	99.5	95.4	107.4

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Ruggedness

Ruggedness of the method was evaluated by performing the standard benzyl cyanide analysis in six replicates using different analyst on different days. The %RSD values of less than 10.0% for Methyl Bromide. These data indicate that the method adopted is rugged. The Ruggedness data and typical overlay chromatograms are presented in **Table 7.**

Table 7: Ruggedness data

Different Days and Analysts		**% RSD**
Day-1	Analyst-1	7.31
	Analyst-2	12.33
	Analyst-1 & 2	10.57
Day-2	Analyst-1	4.91
	Analyst-2	12.33
	Analyst-1 & 2	9.02
Analyst-1	Day-1&2	6.68
Analyst-2	Day-1&2	11.76

Robustness

This study was performed by making small but deliberate variations in the method parameters. The effect of variations in flow rate (0.9 mL/min & 1.1 mL/min) and variation in Injection Volume (0.4µL & 0.6µL) was studied. Under all the variations, system suitability requirement is found to be within the acceptance criteria and hence the proposed method is robust. The relative standard deviation of area counts for Benzyl Cyanide peak obtained from six replicate injections of standard solution should be no more than 10.0%. The data and typical overlay chromatograms of Robustness was as shown in **Table 8**.

Table 8: Robustness data

Name of Imp	**Flow rate (mL/min)**	
	0.9 mL/min (%RSD)	**1.1 mL/min (%RSD)**
Benzyl cyanide	3.73	9.12
	Injection Volume 0.4 (µl)	
	0.4 µL (%RSD)	**0.6 µL (%RSD)**
	2.1	2.95

Solution stability

The 5.0 ppm of Benzyl Cyanide standard solution and Spiked solutions was kept in room temperature. This standard solution was injected duplicates at Initial hours,6 hours and 24 hours. The % of variation in standard and spiked solution should be with in criteria (100±10%). From these results we found that the standard solution of Benzyl Cyanide was stable up to 24 hours. The corresponding data is presented in **Table 9.**

Table 9: Solution stability data

Time period (hours)	% Variation in Standard solution	% Variation in Spiked solution
Initial hours	Not applicable	Not applicable
After 6 h	2.6	-1.8
After 12 h	2.0	-0.7
After 24 h	2.2	4.1

The proposed method was evaluated by the assay of commercially available Primidone tablet for quantification of Benzyl cyanide present in it. The results obtained was compared with the corresponding specification and reported in **Table 10**. This revealed that the content of Benzyl cyanide is not detected in Primidone tablets.

Table 4.12: Tablet analysis

Name of Tablet brand	Label claim (mg)	Benzyl cyanide Content (ppm)
Primidone (Prolet-25)	25	Not detected
Primidone (Prolet-50)	50	Not detected
Primidone (MYSOLINE-250)	250	Not detected

6. Conclusion

A single, rapid and highly selective GC-FID method was developed and validated for the quantification of Benzyl cyanide present in Primidone API through an understanding of LOD, LOQ, nature of stationary phases of columns. The residue Benzyl cyanide was determined in ppm levels also. The method was shown to be specific for Primidone API and was applied successfully to monitor and control impurity level. The method was found to be applicable for the routine analysis of the Primidone API in pharmaceutical industries.

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