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DEVELOPMENT AND VALIDATION OF GC-FID METHOD FOR THE QUANTIFICATION OF 1, 4-DIOXANE IN HERBAL SHAMPOO

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ABSTRACT

1, 4-dioxane is a man-made chemical used primarily as an industrial solvent and also found as organic volatile impurity in cosmetic products. A rapid, simple, accurate and precise method has been developed for the estimation of 1, 4 dioxane in herbal shampoo by gas chromatography coupled with flame ionization detector. Good resolution and sharpness of peak were achieved by using Rtx 5 capillary column (30m x 0.25mm) with stationary phase cross bond 5% diphenyl 95% dimethyl polysiloxane. 1, 4-dioxane was extracted out by simple and rapid liquid-liquid extraction method using methanol and dichloromethane. The amount of 1, 4-dioxane was found 9.32 ppm at retention time 3.52 mins in herbal shampoo. The method validation was carried out with regard to the guidelines for analytical procedures demanded by the International Conference on Harmonization (ICH Q2R1). The lower limit of detection (LOD) and lower quantification limit (LOQ) were obtained 0.10ppm and 0.30ppm respectively. The linearity was over the range of 0.5 ppm to 20 ppm with regression coefficients higher than 0.997. The interday and intraday precision of the method was achieved within 2% relative standard deviation (RSD) with % recoveries between 98 to 102 percent. The robustness of the method done by changing levels of parameters and results were achieved within 2% relative standard deviation (RSD). In specificity study, there was no peak observed at the retention time of the 1, 4-dioxane in placebo.

Keywords: 1, 4-dioxane, Gas chromatography, Herbal shampoo

INTRODUCTION

Dioxane is highly water-soluble. Accordingly, once dioxane forms in water-soluble cosmetics, it is not easily eliminated. It is a byproduct in the production of polyethoxylated surfactants that are widely used in cosmetics.

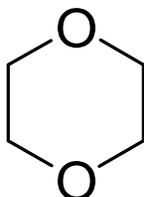


Figure 1: Structure of 1, 4-dioxane

The two mechanisms for 1, 4-dioxane generation are:

- (1) Cyclization reactions during the sulfonation step in alkyl ether sulphates (AES) manufacturing
- (2) The polymerization of ethylene oxide under acidic condition during the manufacture of surfactants.

However, the quantity of 1, 4-dioxane generated by mechanism (2) is expected to be less, because in the most common manufacturing process of alcohol ethoxylate, ethylene oxide is polymerized by mixing it with an alkali catalyst. Ethoxylated surface-active compounds find numerous outlets as washing and/or rinsing agents as well as in the production of cosmetics. These compounds are formed in the ethoxylation reactions wherein a hydrophobic parent substance (alcohol or amine) is treated with oxirane (ethylene oxide - EO).

In herbal shampoo the surfactant ammonium lauryl ether sulphate is used as foaming agent. This is the most potential cleaning agent than any other surfactants. So it is widely used in many shampoos or soaps now a day. 1, 4-dioxane is classified as a Group B2, probable human carcinogenic of low carcinogenic hazard. Target organs effects have been documented for liver, lungs, kidneys and central nervous system. Hence the potential problems associated with 1, 4-dioxane have been brought to the attention of the cosmetic industry and 1, 4-dioxane has been the object of tests in various kinds of cosmetic products.

Literature survey¹⁻¹⁵ revealed that various methods have been reported for estimation of 1, 4-dioxane in raw materials and Pharmaceutical dosage forms. But the sample preparation methods are complex and time consuming or analyzed by using headspace GC and there is no simple method available for the analysis of 1, 4-dioxane in herbal shampoo. Therefore, a sensitive, reliable and rapid method required for estimation of 1, 4-dioxane. Gas chromatography is most widely used for the detection of volatile compounds in the pharmaceutical industry today and 1, 4-dioxane is volatile organic impurity in the herbal shampoo.

MATERIAL AND METHOD

Herbal shampoo (Hair fall control) was procured from Vasu Research Centre, A division of Vasu Healthcare Pvt. Ltd., Vadodara, India.

Standard solutions of 1, 4-dioxane

Preparation of stock solution of 1, 4-dioxane (1000 ppm)

Accurately weighed 25 mg of std. 1, 4-dioxane was transferred in to 25 mL volumetric flask. Diluted up to the mark with dichloromethane.

Preparation of working standard solution of 1, 4-dioxane (100 ppm)

2.5mL aliquot of 1000ppm standard stock solution was transferred in to 25mL volumetric flask and made up to 25mL volume with dichloromethane.

RESULTS

Development and optimization of GC method

(a) Selection of column

Literature survey reveals that for estimation of organic volatile impurities Capillary column is widely used and gives accurate results. Depending on the nature of the 1,4dioxane, the Rtx- 5 column is suitable for the analysis. Rtx- 5 Capillary column having low polarity phase, Crossbond 5% diphenyl 95% dimethyl polysiloxane.

(b) Selection of solvent (Trial 1-6)

1, 4-dioxane is miscible in all the solvents. So, some reported, cheap, easily available and harmless solvents were selected.

Comparison was done between the solvents like Hexane, Methanol, Dichloromethane, Acetone, Toluene and Isobutanol. Accurately weighed 25 mg of std. 1, 4-dioxane was transferred in to tared 25 mL volumetric flask. Diluted up to the mark with Hexane. (1000 ppm) 2.5 mL aliquot of standard stock solution was taken and transferred in to 25 mL volumetric flask. Then diluted up to 25mL with hexane. Preparation was same for all the solvents. Chromatograms were recorded for all the 100 ppm solution of all the solvents (Table 1).

(c) Selection of extraction procedure (Trial 7-18)

From above results of solvents, we selected Hexane, Dichloromethane and Isobutanol for next trials.

Weighed 5 g shampoo in test tube. 2g NaCl and 5mL Methanol were added to it and mixed in Cyclon mixer. Filtered it and in filtrate 10 mL DCM was added. DCM layer was separated out and was passed through anhydrous sodium sulfate to remove moisture and used as test solution (Table 2).

(d) Selection of chromatographic condition (Trial 19-22)

Weighed 5g shampoo in test tube. 2g NaCl and 5mL Methanol were added to it and mixed in Cyclon mixer. Filtered it and in filtrate 10 mL DCM was added. DCM layer was separated out and was passed through

anhydrous sodium sulfate to remove moisture and used as test solution. Chromatographic condition was the initial column temperature fixed at different temperature and time for trials (**Table 3, 4; Figure 2**).

VALIDATION OF DEVELOPED GC METHOD

Developed GC method was validated by different parameters like Specificity, Linearity and range, Accuracy, Precision, LOD, LOQ and Robustness as per ICH guidelines (Q2R1).

Specificity

Specificity of an analytical method is ability to measure specifically the analyte of interest without interferences from blank and placebo. To check specificity, ingredients used in herbal shampoo such as Coco diethanol amid, Coco amido propyl betaine, Coco monoethanolamide, Ethylene glycol mono stearate, Bhringraj, Amalaki, Yashtimadhu, Japa, Shikakai, Methylparaben, Propyl paraben, Poly quaternium 7, D-Panthenol, Cl 19140, perfume, citric acid, ammonium chloride, etc. were mixed in quantity reported in the formulation without addition of the surfactant alkyl lauryl sulphate which is responsible for the presence of 1, 4-dioxane. This placebo was prepared as per the finalized extraction procedure and Chromatographed under developed conditions (**Figure 3-6**).

Linearity and Range

Appropriate volume of aliquots (0.05, 0.1, 0.2, 0.5, 1, 1.5, 2 mL) from working standard of 1, 4 –dioxane containing (100 ppm) were transferred to same volumetric flasks of 10 mL capacity. The volume was adjusted to the mark with diluents (Dichloromethane) to give a solution containing 0.5, 1, 2, 5, 10, 15 and 20 ppm 1, 4 –dioxane. The standard solution was chromatographed for 19.2 min using all developed chromatographic conditions. Graph was plotted for peak area vs. concentration (**Figure 7, 8**).

Limit of Detection (LOD) and Limit of Quantification (LOQ) (Table 5)

Calibration curve was repeated for 6 times and the standard deviation (SD) of the intercepts was calculated. Then LOD and LOQ were measured as follows.

LOD = 3.3 * SD/slope of calibration curve

LOQ = 10 * SD/slope of calibration curve

Where, SD = Standard deviation of intercepts

Precision

Solutions containing different concentrations (lower, middle and higher of linearity range) of 1, 4-dioxane were prepared in dichloromethane. Solutions were injected and chromatograms were recorded. The peak area of 1, 4-dioxane was calculated for each trial. The experiment was repeated 3 times in a day for Intra-day precision (repeatability) and on 3 different days for inter-day precision.

The % RSD of intra-day and inter-day was measured (Table 6, 7).

Accuracy

Accuracy of the method was confirmed by recovery study from sample solution at 3 level of standard addition (80%, 100%, and 120%) of sample solution in triplicate. Chromatogram was recorded and concentration of 1, 4-dioxane was calculated in sample solution called preanalysed solution. Now from sample solution of shampoo, three replicate sample solutions having 9.41ppm of 1,4-dioxane were prepared and 80 %, 100% and 120 % of standard solution of 1, 4-dioxane were spiked in sample solution. Chromatogram was recorded and the % recovery of each aliquot was calculated (Table 8).

Robustness study

To evaluate robustness of the method few parameters were deliberately varied. The parameters included variation of flow rate, oven temperature and column. The change was made at 3 levels and replicate for 3 times. Robustness was checked in terms of RSD value of retention times and peak area of 1, 4-dioxane (Table 9).

ESTIMATION OF 1, 4-DIOXANE IN HERBAL SHAMPOO (Table 10)

Preparation of Standard solutions of 1, 4-dioxane

Preparation of stock solution of 1, 4-dioxane (1000 ppm)

Accurately weighed 25 mg of std. 1, 4-dioxane was transferred in to 25 mL volumetric flask. Diluted up to the mark with dichloromethane.

Preparation of working standard solution of 1, 4-dioxane (100 ppm)

2.5mL aliquot of 1000ppm standard stock solution was transferred in to 25mL volumetric flask and made up to 25mL volume with dichloromethane. This was used as working standard solution.

Preparation of sample solution

Weighed 5 g shampoo in test tube. 2g NaCl and 5mL Methanol were added to it and mixed in Cyclon mixer. Filtered it and in filtrate 10 mL DCM was added. DCM layer was separated out and was passed through anhydrous sodium sulfate to remove moisture and used as test solution. This solution was analyzed by GC and the content of 1, 4-dioxane from herbal shampoo was calculated from following equation,

$$\text{Concentration of sample} = \frac{A_s}{A_t} \times \frac{W_s}{W_t} \times 1000000$$

$$\text{Where, } A_s \text{ is Peak area of standard,}$$

A_t is peak area of test sample,

W_s is Weight of standard,

W_t is Weight of test sample

Table 1: Summary of results of selection of solvent

Trial No.	Solvent	Peak area	R _T	R _S	A _S	N	Remarks
1	Hexane	8266	3.53	12.86	--	64210	Good peak
2	Methanol	5074	3.53	16.39	2.58	37228	Less peak area and high tailing factor
3	DCM	8824	3.54	17.88	--	88200	Good peak. No interference. Good resolution
4	Acetone	6046	3.53	13.24	1.47	46971	Less peak area
5	Toluene	595	3.55	14.28	2.07	82246	Very less peak area
6	Isobutanol	7946	3.53	10.23	1.03	81008	Good peak area

Table 2: Summary of results of trials for extraction procedure

Trial No.	Peak area	RT(min)	Remarks
7	314	3.53	Merged peak and more interference
8	133	3.52	Very less peak area
9	150	3.51	Less peak area
10	204	3.53	Increase the peak area but more interference.
11	356	3.53	Increase the peak area
12	484	3.53	Increase peak area. Good resolution. Less tailing
13	72	3.51	Very less peak area due to evaporation
14	409	3.53	Increase peak area but no recovery
15	575	3.51	Good recovery
16	972	3.52	Good recovery and resolution
16-spiked	625647	3.51	Good recovery
17	480926	3.51	Decrease peak area
18	420896	3.51	Decrease peak area

Table 3: Summary of results of trials for chromatographic conditions

Trial No.	Peak area	RT(min)	Remarks
19	903	4.44	Interference of the other substances
20	764	2.81	Peak merged with the solvent peak
21	480926	3.52	Splitting in the peak due to less hold time
22	420895	3.53	More interference in the spectra. Less resolution

Table 4: Optimized chromatographic condition

Injector temp.	150 °C
Carrier gas	Nitrogen
Injection mode	Split
Pressure	100kpa
Total Flow	50 mL/min
Column	RTX-5(30m x 0.25mm)
Column flow	1 mL/min
Oven temp.	50 °C for 3 min then 50 °C/min increase temp reach up to 110 °C. Hold for 15 min.
Detector temp.	210 °C
Split ratio	30 : 1
Run time	19.20 min



Figure 2: Chromatogram of 1,4-dioxane in shampoo

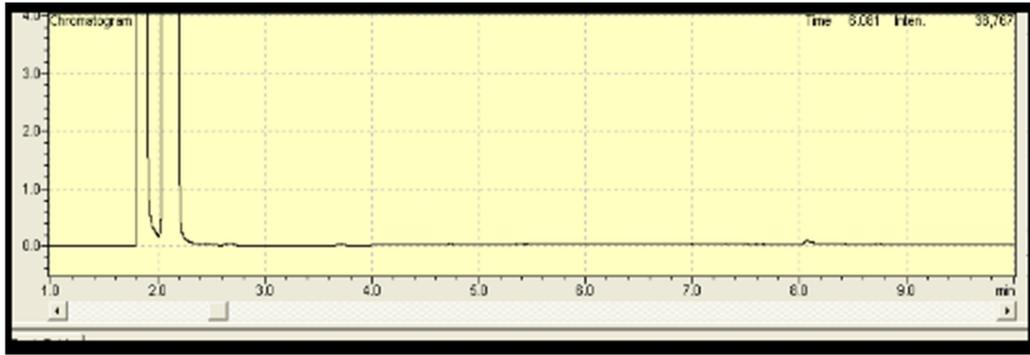


Figure 3: Chromatogram of blank (DCM)



Figure 4: Chromatogram of placebo

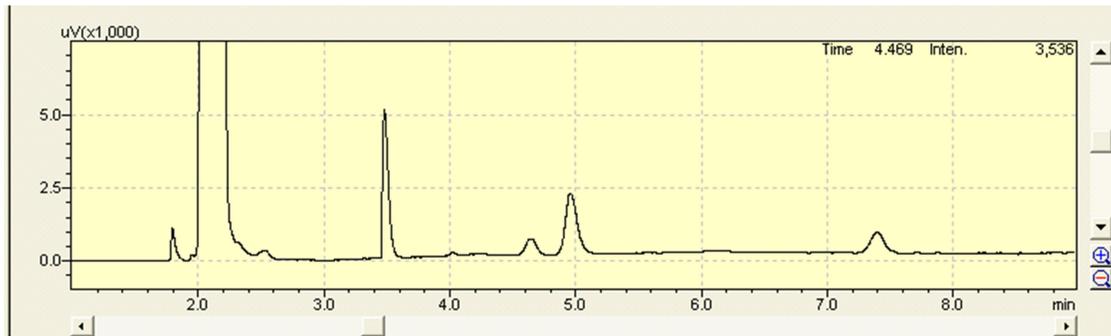


Figure 5: Chromatogram of working standard solution of 1,4-dioxane(100ppm)



Figure 6: Chromatogram of Sample

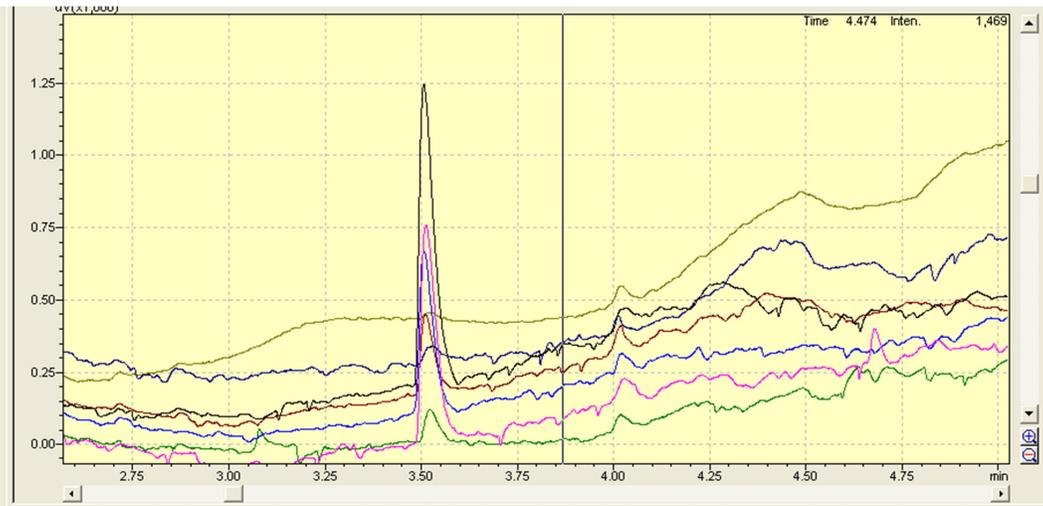


Figure 7: Linearity chromatograph of 1, 4-dioxane

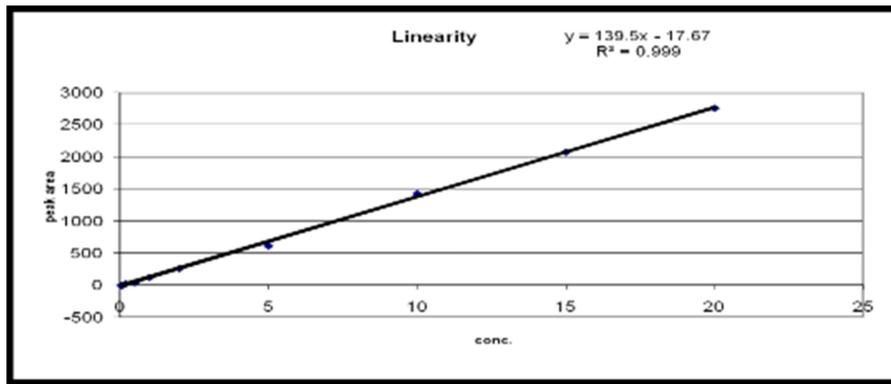


Figure 8: Calibration curve of 1, 4-dioxane

Table 5: LOD & LOQ

LOD	LOQ	SD
0.10 ppm	0.30 ppm	4.51

Table 6: Intraday precision data of 1, 4-dioxane

Concentration(ppm)	Peak area			Mean	SD (n=3)	%RSD
	Set 1	Set 2	Set 3			
0.5	41	40	40	40.333	0.57	1.43
5	720	729	727	725.33	4.72	0.65
20	3019	3009	3028	3018.7	9.5	0.31

Table 7: Inter day precision data of 1, 4-dioxane

Concentration(ppm)	Peak area			Mean	SD (n=3)	%RSD
	Day 1	Day 2	Day 3			
0.5	38	38	39	38.33	0.57	1.50
5	720	715	726	720.33	5.50	0.76
20	3019	3028	2996	3014.3	16.50	0.54

Table 8: Recovery study of 1, 4-dioxane (n=3)

Level of recovery	Amt of 1, 4-dioxane in sample (ppm)	Amt of 1,4 dioxane added (ppm)	Total peak area	Total Amt. Found (ppm)	Amt recovered (ppm)	% Recovery	Mean % recovery \pm SD
80%	9.41	7.52	1382	16.62	7.21	95.87	98.22 \pm 2.26
	9.41	7.52	1393	16.81	7.40	98.40	
	9.41	7.52	1407	16.96	7.55	100.39	
100%	9.41	9.41	1604	19.34	9.93	105.52	101.25 \pm 4.51
	9.41	9.41	1514	18.49	9.08	96.52	
	9.41	9.41	1581	18.98	9.57	101.70	
120%	9.41	11.29	1803	21.48	12.07	106.90	99.97 \pm 6.30
	9.41	11.29	1722	20.52	11.11	98.40	
	9.41	11.29	1686	20.09	10.68	94.59	

Table 9: Robustness study (n=3)

Parameters	Percentage Change	Mean \pm SD (RT)	%RSD (RT)	Mean \pm SD (PA)	% RSD(PA)
Column	Column 1	3.50 \pm 0	0.00	1438 \pm 7.94	0.55
	Column 2	3.53 \pm 0.01	0.28	1380.67 \pm 14.01	1.01
Oven temperature	40 °C (-20%)	3.94 \pm 0.01	0.15	1385.33 \pm 19.55	1.41
	50 °C	3.50 \pm 0	0.00	1438 \pm 7.94	0.55
	60 °C (+20%)	3.11 \pm 0.02	0.49	1293.33 \pm 18.58	1.44
Flow rate	1.1 ml/min (10%)	3.42 \pm 0.02	0.45	1266.33 \pm 15.82	1.25
	1 ml/min	3.50 \pm 0	0.00	1438 \pm 7.94	0.55
	0.9 ml/min (-10%)	3.58 \pm 0.01	0.28	1334.33 \pm 24.65	1.84

Table 10: Concentration of 1, 4-dioxane in herbal shampoo

	Peak area	Concentration (ppm)	Mean(n=3)
1, 4-dioxane	763	9.33	9.32 \pm 0.065
	758	9.25	
	771	9.38	

DISCUSSION

Chromatogram of placebo showed that no peak at retention time of 1,4dioxane i.e.3.52 min. So this developed method is specific for the estimation of 1,4dioxane. Linearity data of 1, 4-dioxane were predict Regression co-efficient- 0.999 and regression equation was found $y= 139.5x-17.67$. The linear range for 1, 4-dioxane was found from 0.5 to 20 ppm. % RSD was less than 2, which indicates that the proposed method is repeatable and precise. Result obtained from accuracy study reveals that % recovery of 1, 4-dioxane was within acceptance criteria given in ICH i.e. 98-102%. The robustness study suggested

that all the parameters have no significant influence on the determination. Results indicate that the selected factors remained unaffected by small variation of these parameters and % RSD was less than 2, which demonstrates that the proposed method was robust.

CONCLUSION

The proposed GC-FID method for the **Quantification of 1, 4-Dioxane in Herbal Shampoo** was found to be sensitive, accurate, precise, simple, and rapid. Hence the present GC-FID method may be used for routine analysis of **1, 4-Dioxane in Herbal Shampoo**.

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