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IMPURITY PROFILING OF OXYBENZONE BY RP-HPLC METHOD

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ABSTRACT

A simple, accurate, economical and reproducible reverse phase high performance liquid chromatographic (RP-HPLC) method was developed for the determination of Ben-1, Benzophenone, Benzyl trichloride and Resorsinol in Oxybenzone. The separation was achieved on a kromosil C18 column (150 × 4.6 mm i.d, particle size of 5 µ) using a mixture of Formic Acid(0.2%) and acetonitrile in the ratio of 80:20 %v/v as mobile phase in an isocratic elution mode, at a flow rate of 1ml/min. The detection was monitored at 250nm for Ben-1, Benzophenone, Benzyl trichloride and Resorsinol. The retention time of Ben-1, Benzophenone, Resorsinol and Benzyl trichloride were found to be 15.738, 18.682, 2.747 and 8.328 mins respectively. The method was validated for Recovery studies. Method was successfully applied for the determination of Ben-1, Benzophenone, Benzyl trichloride and Resorsinol in Oxybenzone sample.

KEYWORDS: Ben-1, Benzophenone, Benzyl trichloride, Resorsinol Oxybenzone, RP-HPLC method.

INTRODUCTION

Oxybenzone or Benzophenone-3 is chemically(2-hydroxy-4-methoxyphenyl) - phenylmethan one an organic compound used in sunscreens. Oxybenzone belongs to the class of aromatic ketones known as benzophenones. It is a Topical Sunscreen Agent providing UVA/UVB coverage and approved for use by the FDA at concentrations up to 6%. Benzophenone-3 (BZ-3) is a category 1 (over-the-counter) product approved by the US Food and Drug Administration (FDA) for use as a sunscreen agent in medicine, cosmetics,

industry, and agriculture. As a photo protective agent, it has an absorption profile spanning from 270 to 350 nm. It is one of the most widely used organic UVA filters in sunscreens today. It is also found in nail polish, fragrances, hairspray, and cosmetics as a photo stabilizer. Oxybenzone has been a FDA approved sunscreen agent since 1980. Oxybenzone works by absorbing UV radiations within a specific wavelength range, it diminish the penetration of UV light through the epidermis by dispelling it as heat. Previous studies have shown that oxybenzone penetrates the skin, and it can be found in urine, feces, and blood. In a study the amount of oxybenzone absorbed was measured in urine, as experimental studies in the rat have shown that urine is the major route of excretion. Eleven volunteers applied the recommended amount of a commercially available sunscreen and urine samples were collected during a 48-hr period after application. The average total amount excreted was 11 mg, median 9.8 mg, which is approximately 0.4% of the applied amount of oxybenzone. Some of the volunteers still excreted oxybenzone 48 hr after application. The chemical structure of Oxybenzone is represented in figure 1.

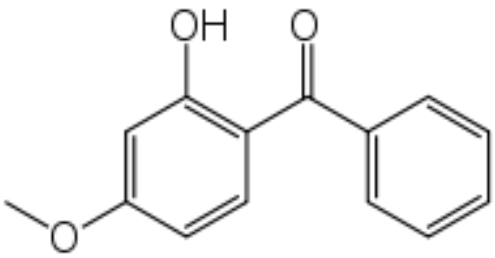

Figure 1 Chemical structure of Oxybenzone.

Several methods for the analysis of the Oxybenzone are developed such as Method development and validation for simultaneous estimation of Oxybenzone, Octinoxate and Avobenzone in sunscreen lotion by reverse phase high performance liquid chromatography (Banker et.al 2011), Determination of UV-filters in sunscreens by HPLC (Chisvert et.al 2001), Simultaneous quantitative estimation of oxybenzone and 2-ethylhexyl-4-methoxycinnamate in sunscreen formulations by second order derivative spectrophotometry (H. M Chawla et. al 2009). However method for impurity profiling is not yet developed though the raw materials used in the synthesis of Oxybenzone belongs to a class of Benzophenones, which are carcinogenic in nature if present in the excess limits.

Therefore taking into account a method has been develop to estimate the raw material impurities in Oxybenzone sample. The advantage of this method is raw materials which may carry in the final product of Oxybenzone be quantified and checked within the limit.

MATERIALS AND METHODS

Materials: All the materials used in the research work were provided by Vivimed laboratories limited. Benzophenone, Ben-1, Benzyl trichloride and Resorsinol sigmaldrich standards were used, oxybenzone sample was procured from the vivimed manufacturing unit. Formic acid, HPLC grade methanol and acetonitrile of Merck Millipore was used. Distilled water was used throughout the analysis.

Instrumentation: A high performance liquid chromatography system (Shimadzu LC 2010HT) was used fitted with a PDA detector and LC solution software

Preparation of solutions

For Retention time: 12.5mg each of Benzophenone, Ben-1, Benzyl trichloride and Resorsinol were individually weighted in 25ml volumetric flask and made up the volume using methanol as a solvent.

Mixed standard impurities solution: The solution of mixed standard impurities was prepared by dissolving 25.55mg of Benzophenone, 25.47mg of Ben-1, 25.77mg of Benzyl trichloride and 25.10mg of Resorsinol in 50ml volumetric flask and made up the volume using methanol as a solvent (solution A). This solution was further diluted with methanol, to attain a concentration of 1000ppm with respect to sample (solution B).

Preparation of sample solution: Sample solution was prepared by weighing 125.36mg of Oxybenzone sample in a 25ml volumetric flask, the volume was made up to the mark using methanol as a solvent.

Spiked sample preparation: Sample solution of concentration that was used for analysis of sample was prepared as mentioned in preperation of sample solution section. To this 2.5ml of the standard solution B was added, this resulted in 100ppm of mixed standard impurities in spiked sample.

Chromatographic Conditions: The mobile phase consisted of 0.2% Formic acid and Acetonitrile (80:20 v/v). The mobile phase was isocratically pumped at a flow rate of

1ml/min. The analytical Kromasil C18 column(150 × 4.6 mm i.d 5 µm) was used as a stationary phase for the chromatographic separation. The mobile phase was filtered under vacuum through 0.45 µm nylon membrane filter (Whatman International, England) and degassed before use.

METHOD DEVELOPMENT

Detection wavelength for the HPLC studies was selected as 250nm after recording the UV spectrum from 190 to 800nm of the mixed standard impurities and oxybenzone by using PDA detector HPLC. The suitable area and peak selectivity of the standard impurities and oxybenzone was observed at this wavelength. The chromatographic conditions were optimized for the resolution of peaks of the standard impurities and oxybenzone under each conditions by varying the stationary phase, proportion of the Water, Acetonitrile and Methanol in the mobile phase and flow rate using the representative samples. Several trials using various proportions of Water, Acetonitrile and Methanol were carried out. However to attain the selectivity, resolution of the standard impurities and oxybenzone, formic acid was introduced as a 0.2%. subsequently a mixture of mobile phase compositions was used to optimise the chromatographic conditions for resolving standard impurities and oxybenzone in a single run. An appropriate blank was selected as methanol and injected before the analysis of the samples. A steady baseline was recorded with the fixed chromatographic conditions. Mixed standard impurities solution of concentration 1000ppm with respect to sample was injected twice to record the chromatograms of standard. This was followed by injections of sample solution and chromatograms were recorded. Such an optimized method was then used to study the impurities in the oxybenzone sample.

The content of impurities in sample can be calculated by using below formula:

$$\text{A (content in ppm)} = \frac{\text{Average area of sample} \times \text{std conc} \times 100000}{\text{Average area of standard} \times \text{sample conc}}$$

Recovery studies: Recovery studies were performed to know the reproducibility of method. Sample solution of concentration that was used for analysis of sample was prepared as mentioned in analysis of sample section. This sample solution was spiked with standard 100ppm of Ben-1, Benzophenone, Benzyl trichloride and Resorsinol was injected to record chromatograms.

The content of impurities in spiked sample can be calculated by using below formula:

$$A \text{ (content in ppm)} = \frac{\text{Average area of sample} \times \text{std conc} \times 100000}{\text{Average area of standard} \times \text{sample conc}}$$

RESULTS AND DISCUSSION

The chromatograms were recorded for each relative substance by optimized chromatographic conditions using methanol as solvent and retention time in mins were found as below.

- Ben-1 – 15.738
- Benzophenone – 18.682
- Resorsinol – 2.747
- Benzyl trichloride – 8.328

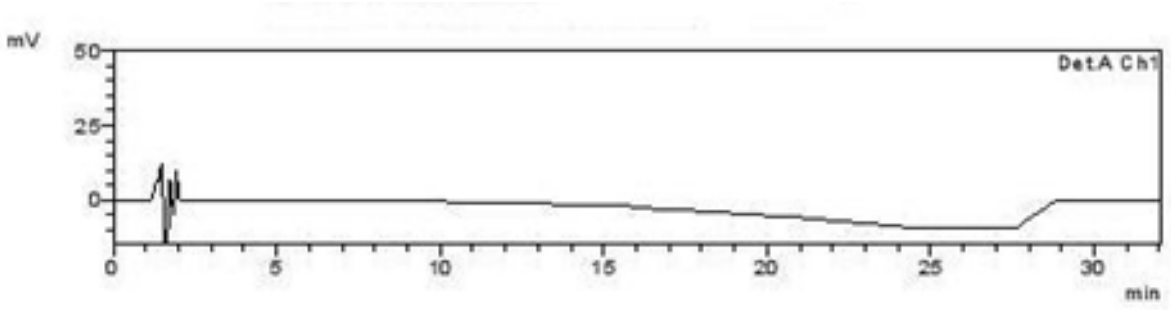

Figure 2 Chromatogram of Blank (Methanol).

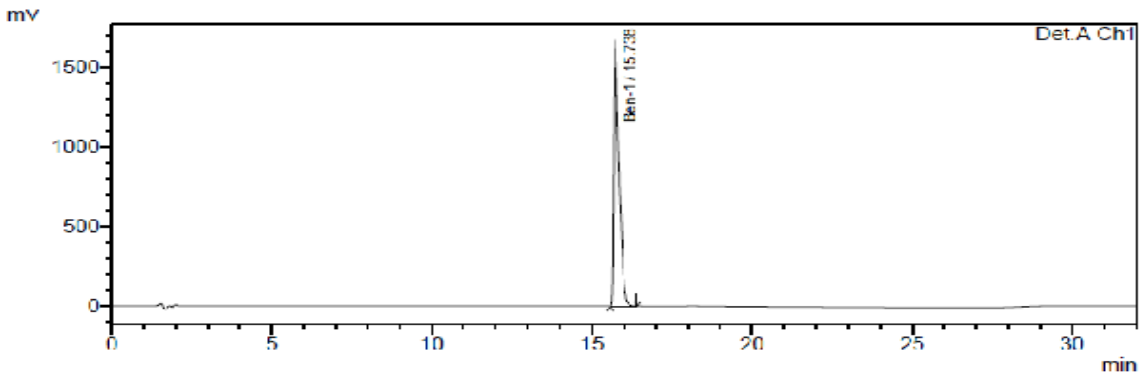

Figure 3 Chromatogram Showing Retention Time of Ben-1.

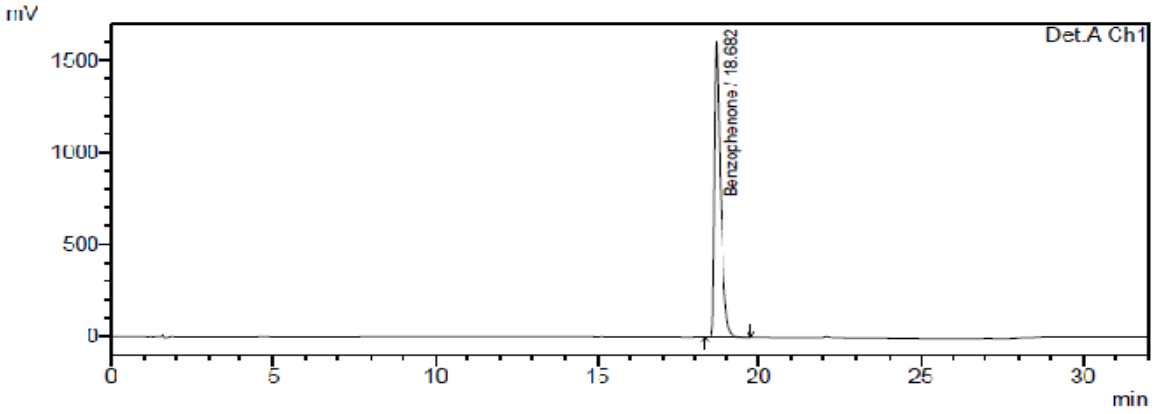

Figure 4 Chromatogram showing Retention Time of Benophenone.

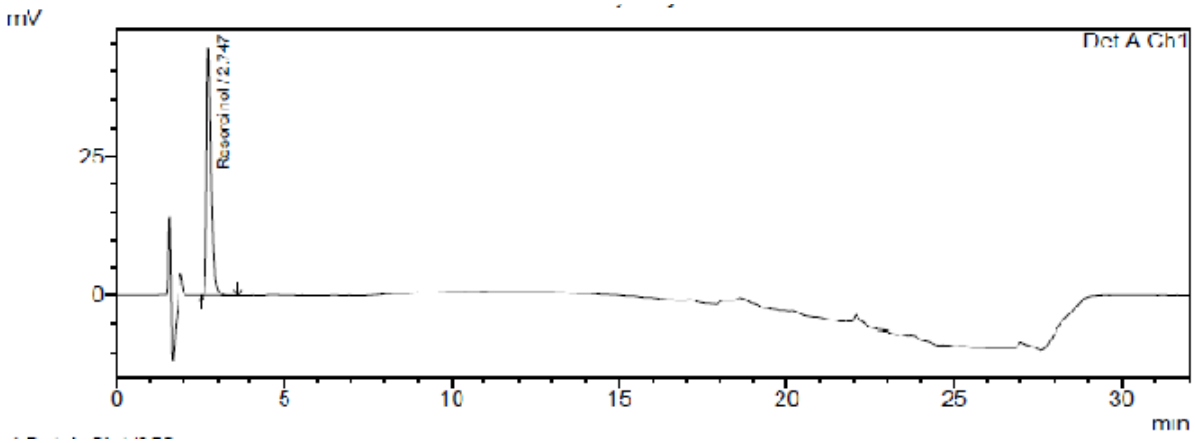

Figure 5 Chromatogram showing Retention Time of Resorsinol.

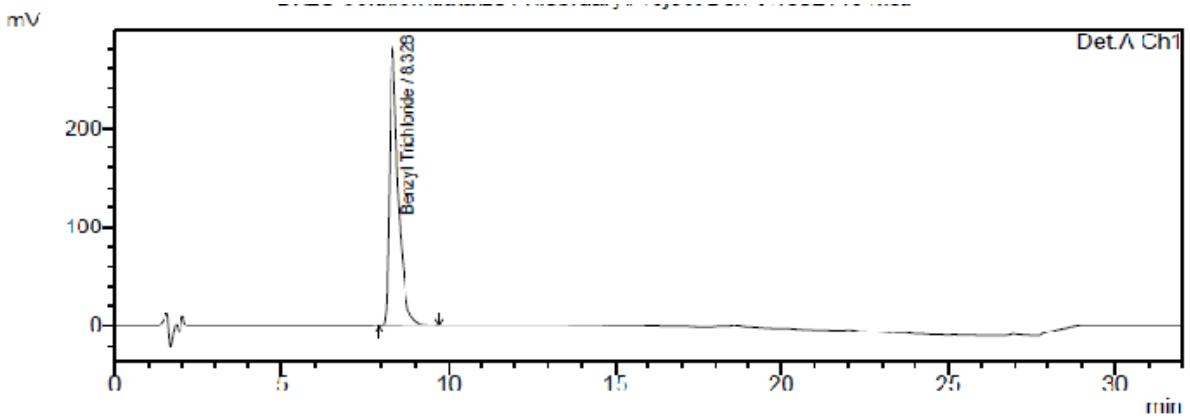

Figure 6 Chromatogram showing Retention Time of Benzyl trichloride.

Recording chromatograms of the mixed standard impurities and sample injection. A steady baseline was recorded with the fixed chromatographic conditions. Solution B was injected twice to record the chromatograms of standard.

This was followed by injections of sample solution and chromatograms were recorded.

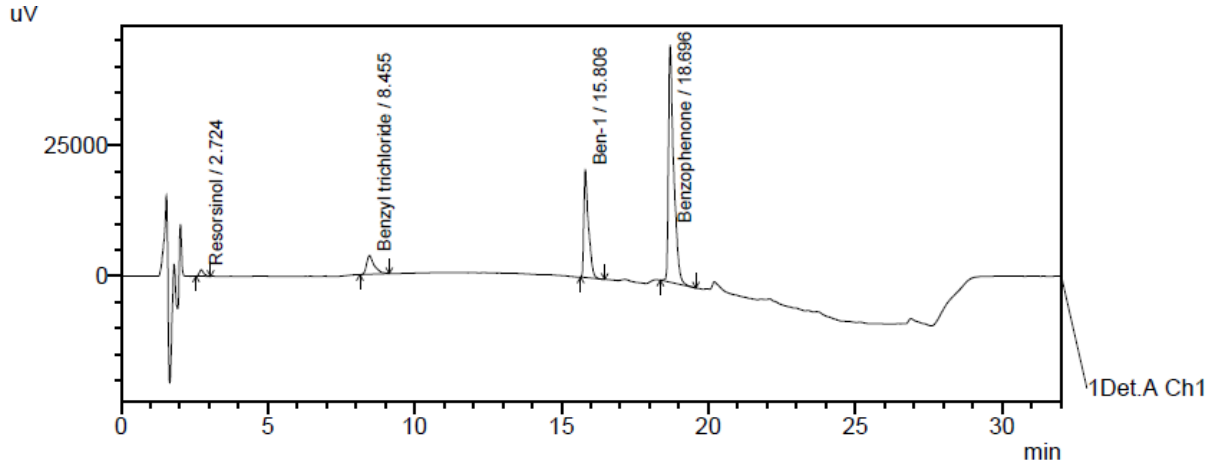

Figure 7 Chromatogram Showing Injection-1 of Standard impurities.

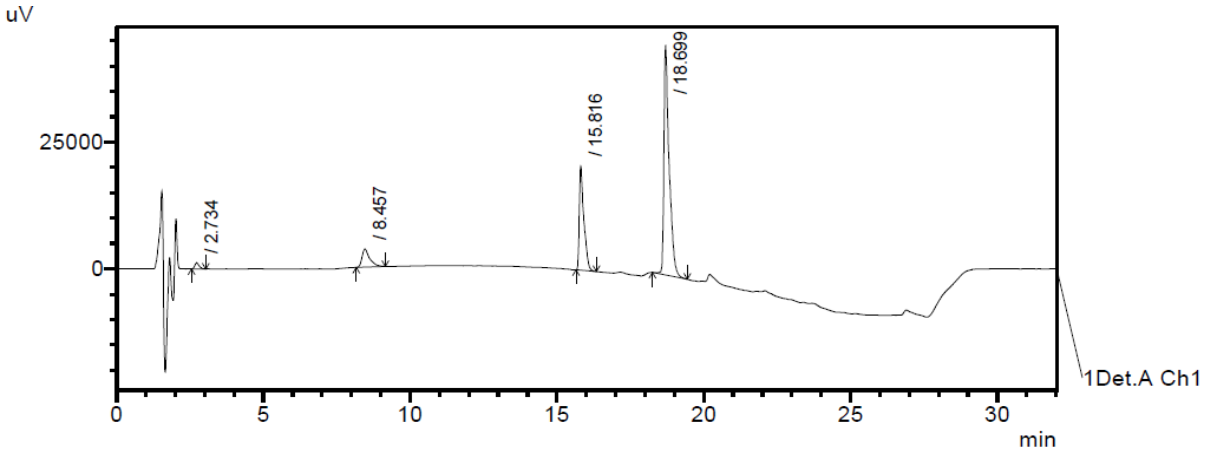

Figure 8 Chromatogram Showing Injection-2 of Standard impurities.

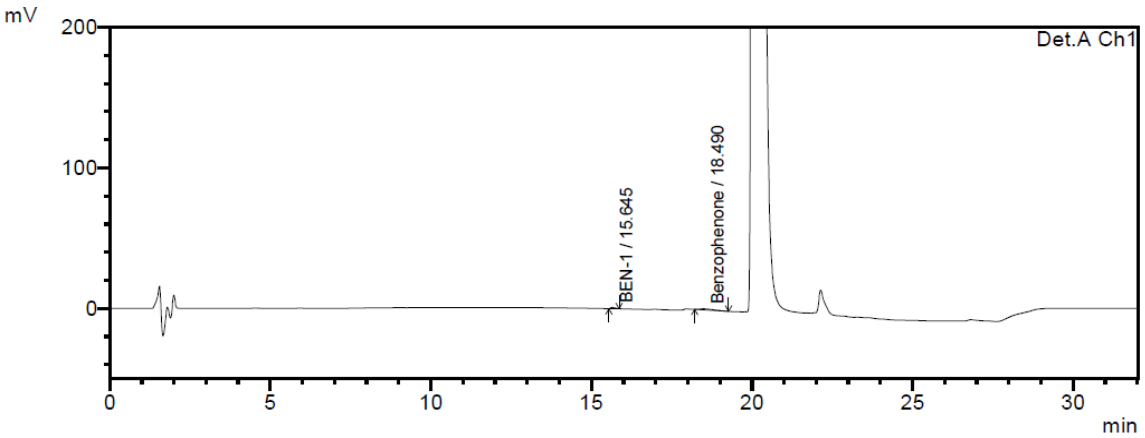

Figure 9 Chromatogram Showing Injection-1 of Sample.

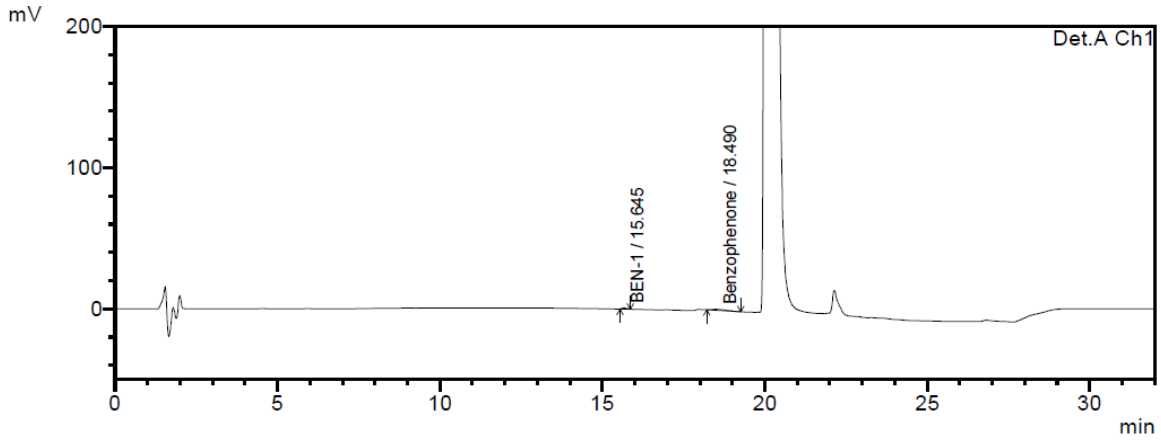

Figure 10 Chromatogram Showing Injection-2 of Sample.

Only Ben-1 and Benzophenone were detected in the sample which can be seen in Fig, 9 and 10 their content can be calculated as below

Ben-1 calculation

Table 1

S. No	Injection Number	Name	Area
1	1	Std.Inj No:1	222387
2	2	Std.Inj No:2	222070
		Mean	222229
		Std.Deviation	224.153
		% RSD	0.10087

Table 2

S. No	Injection Number	Name	Area
1	1	Sample.Inj No:1	8113
2	2	Sample.Inj No:2	8210
		Mean	8161.5
		Std.Deviation	68.5894
		% RSD	0.8404

Table 3

			Dilution(ml)	Ml	ml
Std.Wt	Mg	25.47	50	1	100
Sample.Wt	Mg	125.33	25		

$$\text{Ben-1 (ppm)} = \frac{\text{Avg area of Ben-1 in sample} \times \text{std conc} \times 100000}{\text{Avg area of Ben-1 in standard} \times \text{sample conc}}$$

$$= 37.31 \text{ ppm}$$

Benzophenone calculation

Table 4

S. No	Injection Number	Name	Area
1	1	Std.Inj No:1	564291
2	2	Std.Inj No:2	564560
		Mean	564426
		Std.Deviation	190.212
		% RSD	0.0337

Table 5

S. No	Injection Number	Name	Area
1	1	Sample.Inj No:1	22397
2	2	Sample.Inj No:2	22306
		Mean	22351.5
		Std.Deviation	64.3467
		% RSD	0.28987

Table 6

			Dilution(ml)	Ml	ml
Std.Wt	Mg	25.47	50	1	100
Sample.Wt	Mg	125.33	25		

$$\text{Benzophenone (ppm)} = \frac{\text{Avg area of Benzophenone in sample} \times \text{std conc} \times 100000}{\text{Avg area of Benzophenone standard} \times \text{sample conc}}$$

$$= 40.36 \text{ ppm}$$

Recovery studies

Recovery studies were performed to check the reproducibility of method. Sample solution of concentration that was used for analysis of sample was prepared as mentioned in preperation of sample solution section. This sample solution was spiked with standard 100ppm of Ben-1, Benzophenone, Benzyl trichloride and Resorsinol was injected to record chromatograms.

Recording of chromatograms

A steady baseline was recorded with the fixed chromatographic conditions. Spiked sample solution was injected to record the chromatogram of spiked sample, Fig. 7.4.1 to 7.4.2

The content of impurities in spiked sample can be calculated by using below formula:

$$\text{A (content in ppm)} = \frac{\text{Average area of spiked sample} \times \text{std conc} \times 100000}{\text{Average area of standard} \times \text{sample conc}}$$

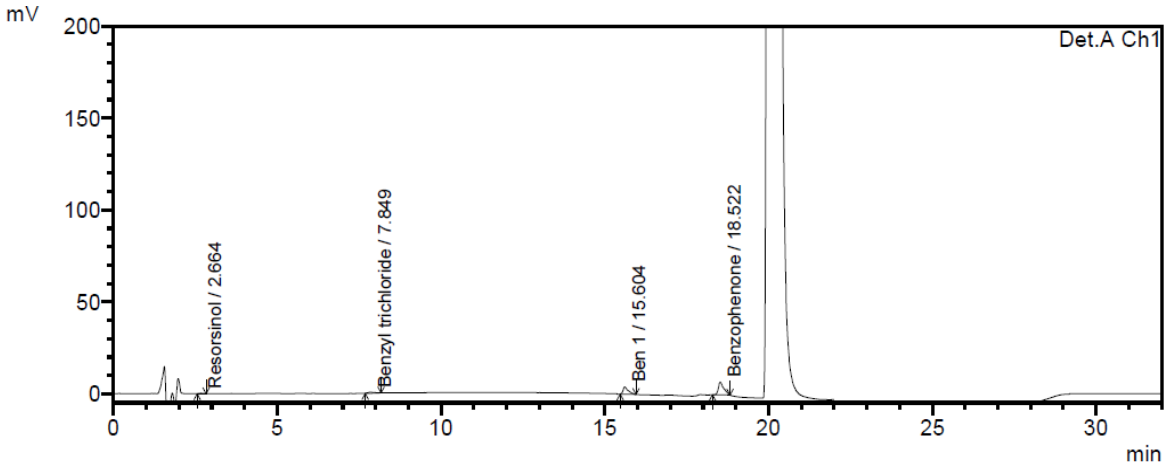

Fig 11 Chromatogram Showing Injection-1 of Spiked Sample.

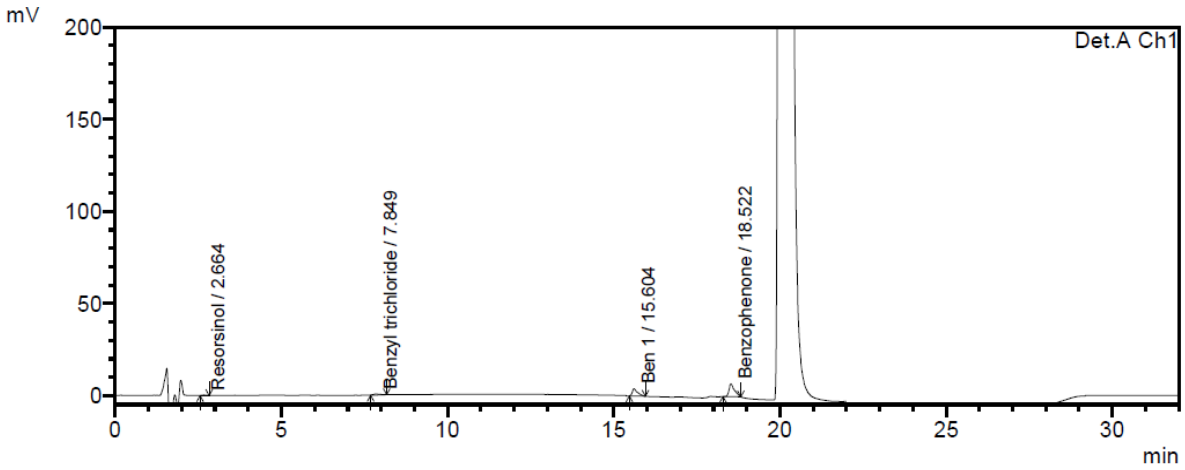

Fig 12 Chromatogram Showing Injection-2 of Spiked Sample.

BEN-1 Content in Spiked Sample

Table 7

S. No	Injection Number	Name	Area
1	1	Std.Inj No:1	564291
2	2	Std.Inj No:2	564560
		Mean	564425.5
		Std.Deviation	190.21172
		% RSD	0.0337001

Table 8

S. No	Injection Number	Name	Area
1	1	Sample.Inj No:1	40915
2	2	Sample.Inj No:2	40957
		Mean	40936
		Std.Deviation	29.698485
		% RSD	0.0725486

Table 9

			Dilution(ml)	Ml	ml
Std.Wt	Mg	25.55	50	1	100
Sam.Wt	Mg	250.01	50		

$$\text{Ben-1 (ppm)} = \frac{\text{Avg area of Ben-1 in sample} \times \text{std conc} \times 100000}{\text{Avg area of Ben-1 in standard} \times \text{sample conc}}$$

= 139 ppm

Benzophenone Content In Spiked Sample

Table 10

S. No	Injection Number	Name	Area
1	1	Std.Inj No:1	564291
2	2	Std.Inj No:2	564560
		Mean	564425.5
		Std.Deviation	190.2117241
		% RSD	0.033700059

Table 11

S. No	Injection Number	Name	Area
1	1	Sample.Inj No:1	79133
2	2	Sample.Inj No:2	78109
		Mean	78621
		Std.Deviation	724.07734
		% RSD	0.920971934

Table 12

			Dilution(ml)	Ml	Ml
Std.Wt	Mg	25.55	50	1	100
Sam.Wt	Mg	250.01	50		

$$\text{Benzophenone (ppm)} = \frac{\text{Avg area of Benzophenone in sample} \times \text{std conc} \times 100000}{\text{Avg area of Benzophenone in standard} \times \text{sample conc}}$$

= 142.35 ppm

Resorsinol Content In Spiked Sample

Table 13

S. No	Injection Number	Name	Area
1	1	Std.Inj No:1	12232
2	2	Std.Inj No:2	12201
		Mean	12216.5

		Std.Deviation	21.9203
		% RSD	0.17943

Table 14

S. No	Injection Number	Name	Area
1	1	Sample.Inj No:1	1491
2	2	Sample.Inj No:2	1447
		Mean	1469
		Std.Deviation	31.1127
		% RSD	2.11795

Table 15

			Dilution(ml)	Ml	Ml
Std.Wt	Mg	25.55	50	1	100
Sam.Wt	Mg	250.01	50		

$$\text{Resorsinol (ppm)} = \frac{\text{Avg area of Resorsinol in sample} \times \text{std conc} \times 100000}{\text{Avg area of Resorsinol in standard} \times \text{sample conc}}$$

= 102.89 ppm

Benzyl trichloride Content In Spiked Sample

Table 16

S. No	Injection Number	Name	Area
1	1	Std.Inj No:1	65180
2	2	Std.Inj No:2	65240
		Mean	65210
		Std.Deviation	42.4264
		% RSD	0.06506

Table 17

S. No	Injection Number	Name	Area
1	1	Sample.Inj No:1	6677
2	2	Sample.Inj No:2	6307
		Mean	6492
		Std.Deviation	261.63
		% RSD	0.12662

Table 18

			Dilution(ml)	ml	Ml
Std.Wt	Mg	25.55	50	1	100
Sam.Wt	Mg	250.01	50		

$$\text{Benzyl Trichloride (ppm)} = \frac{\text{Avg area of Benzyl trichloride in sample} \times \text{std conc} \times 100000}{\text{Avg area of Benzyl trichloride in standard} \times \text{sample conc}}$$

= 101.74 ppm

CONCLUSION

For routine analytical purpose, it is always necessary to establish methods capable of analyzing huge number of samples in short time with due accuracy. In the present work attempt was made to develop a new method for determination of Benzophenone, Ben-1, Resorsinol and Benzyl trichloride in Oxybenzone and validate it for accuracy and applying the same for its estimation in Oxybenzone sample.

In Oxybenzone sample only Benzophenone and Ben-1 were detected and their content was found to be 40.36 and 37.31ppm respectively.

The Recovery results obtained for this method were promising. Hence the developed method can be adopted for determination of Benzophenone, Ben-1, Resorsinol and Benzyl trichloride, in Oxybenzone in quality control laboratories.

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