WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 4, Issue 2, 510-523. Research Article ISSN 2277– 7105

IMPURITY PROFILING OF SULISOBENZONE BY RP-HPLC METHOD

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Article Received on
12 Nov 2014,

Revised on 07 Dec 2014,
Accepted on 01 Jan 2015

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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 Benzophenone, Ben-1, Ben-3 in Sulisobenzone. 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 65:35 %v/v as mobile phase in an isocratic elution mode, at a flow rate of 1 ml/min. The detection was monitored at 290nm for Ben-1, Ben-3 and at 250nm for Benzophenone. The retention time of Benzophenone, Ben-1, Ben-3 were found to be 9.116, 15.564, 13.370 mins respectively. The method was validated for Recovery studies. Method was successfully applied for the determination of Benzophenone, Ben-1, Ben-3 in Sulisobenzone sample.

Keywords: Benzophenone, Ben-1, Ben-3, Sulisobenzone, RP-HPLC method.

INTRODUCTION

Sulisobenzone or benzophenone-4 is chemically5-Benzoyl -4-hydroxy -2-methoxy benzenesulfonic acidan organic compound used in sunscreens. Sulisobenzone belongs to the class of aromatic ketonesknown as benzophenones. It is aTopical Sunscreen Agent providing UVA/UVB coverage and approved for use at concentrations up to 5% by FDA and at 6% in Canada by Health Canada. As aphoto protective agent, it has an absorption profile spanning from 290 to 360 nm. Sulisobenzone 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.The chemical structure ofSulisobenzone is represented in figure 1.

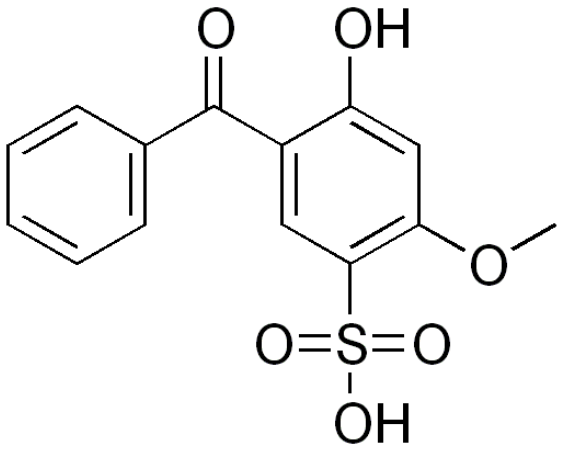

Figure 1: Chemical structure of Sulisobenzone.

Several methods for the analysis of the Sulisobenzone are developed such as Method development for quantitative determination of four preservatives and nine UV filters worldwide authorized in commercial suncare product by reverse phase high performance liquid chromatography (Kicheol Kim 1* and et al),Determination of UV-filters in sunscreens by HPLC (Chisvert et.al 2001). However method for impurity profiling is not yet developed though the raw materials used in the synthesis of Sulisobenzone 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 Sulisobenzone sample. The advantage of this method is raw materials which may carry in the final product of Sulisobenzone 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, Ben-3sigmaldrich standards were used, Sulisobenzone sample was procured from the Local laboratory. 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, and Ben-3 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 25.47mg of Benzophenone, 25.47mg of Ben-1, 25.41mg of Ben-3 in 50ml volumetric flask and made up the volume using methanol as a solvent. This solution was further diluted with methanol, to attain a concentration of 1000ppm with respect to sample.

Preparation of sample solution

Sample solution was prepared by weighing 125.33mg of Sulisobenzone 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 analysis of sample section. To this 0.05ml of the standard solution A was added, this resulted in 200ppm of mixed standard impurities in spiked sample.

Chromatographic Conditions

The mobile phase consisted of 0.2% Formic acid and Acetonitrile (65:35 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 5µm) was used as a stationary phase for the chromatographic separation. The mobile phase was filtered under vacuum through 0.45µm nylon membrane filter (Whatman International, England) and degassed before use.

Method Development

Detection wavelength for the HPLC studies was selected as 250nm for Benzophenone and 290nm for Ben-1, Ben-3 after recording the UV spectrum from 190 to 800nm of the mixed standard impurities and Sulisobenzone by using PDA detector HPLC. The suitable area and peak selectivity of the standard impurities and Sulisobenzone was observed at this

wavelengths. The chromatographic conditions were optimized for the resolution of peaks of the standard impurities and Sulisobenzone under each condition 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 Sulisobenzone, 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 Sulisobenzone 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 Sulisobenzone sample.

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

$$A \text{ (content in ppm)} = \frac{\text{Average area of sample} \times \text{stdconc} \times 1000000}{\text{Average area of standard} \times \text{sample conc}}$$

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 200ppm of Ben-1, Benzophenone, and Ben-3 and was injected to record chromatograms.

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

$$A \text{ (content in ppm)} = \frac{\text{Average area of sample} \times \text{stdconc} \times 1000000}{\text{Average area of standard} \times \text{sample conc}}$$

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

1. Benzophenone – 13.370 min (250 nm)
2. Ben-1 – 9.116 min (290 nm)
3. Ben-3 – 15.564 min(290 nm)

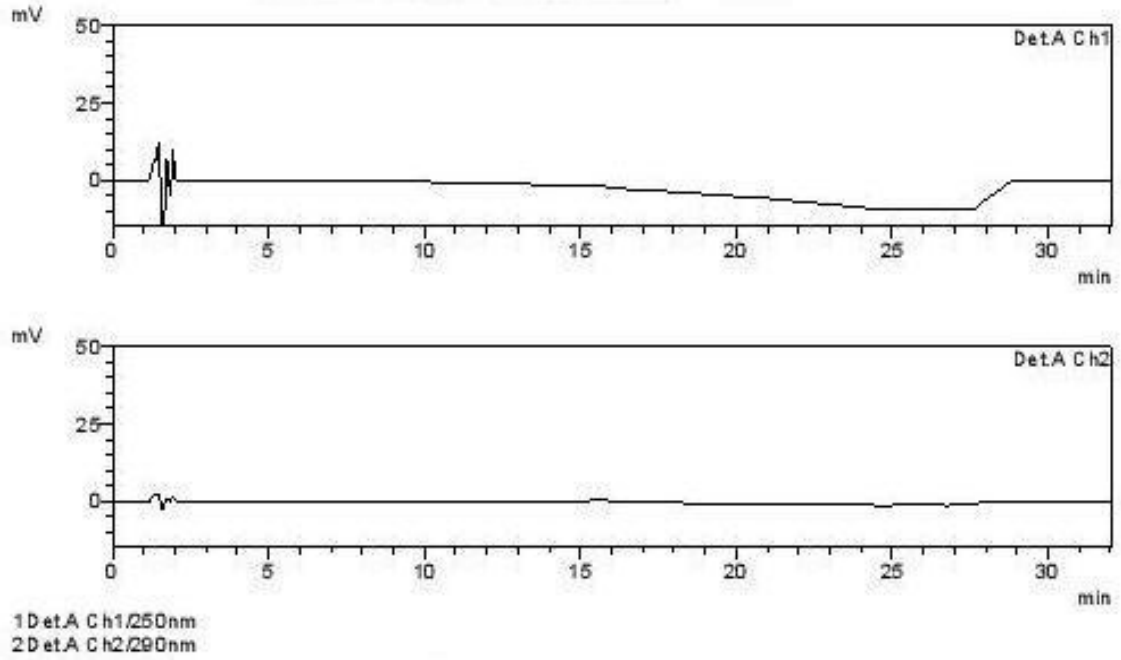

Figure 2: Chromatogram of Blank (Methanol).

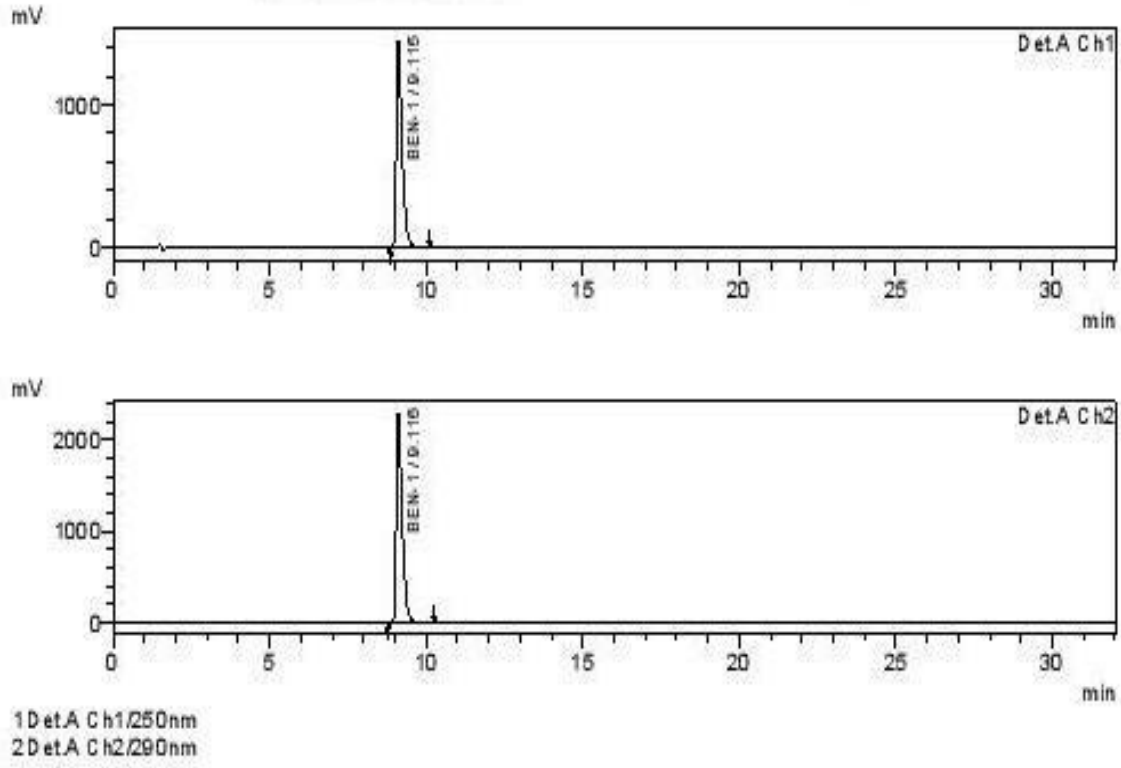

Figure 3: Chromatogram Showing Retention Time of Ben-1.

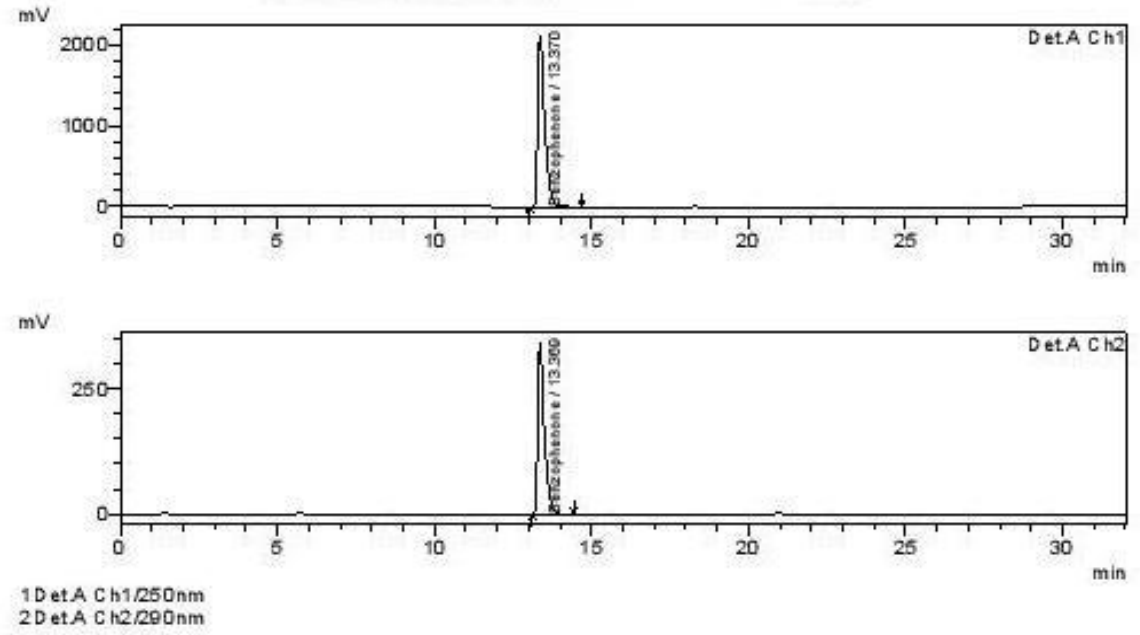

Figure 4: Chromatogram showing Retention Time of Benophenone.

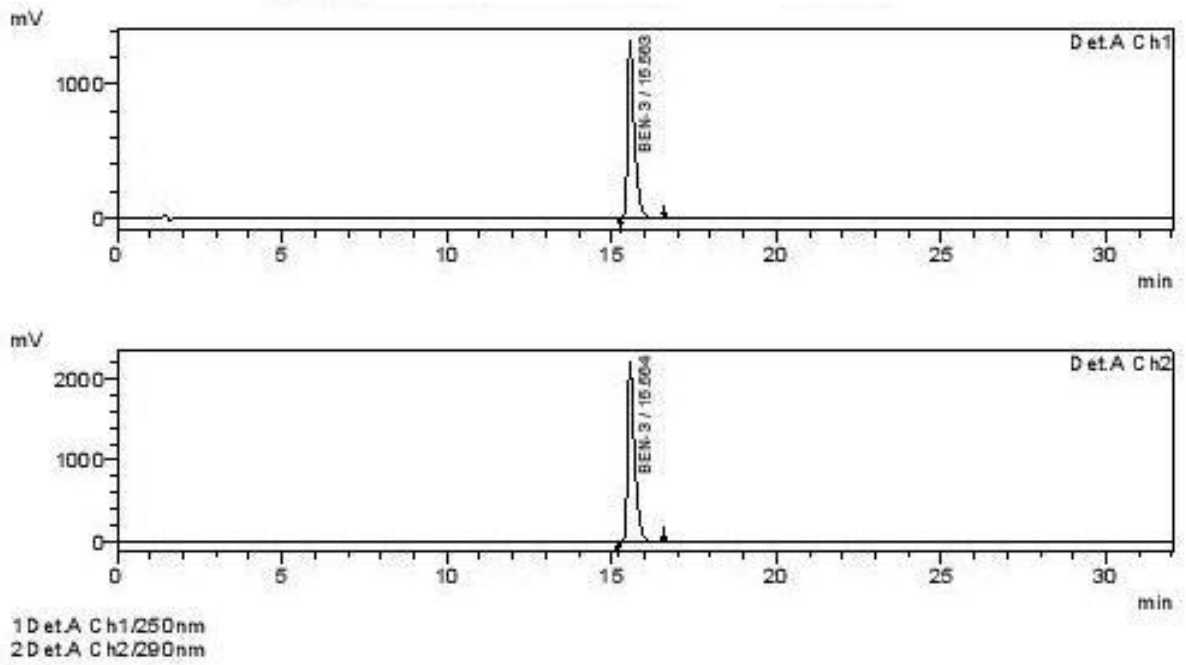

Figure 6: Chromatogram showing Retention Time of Ben-3.

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.

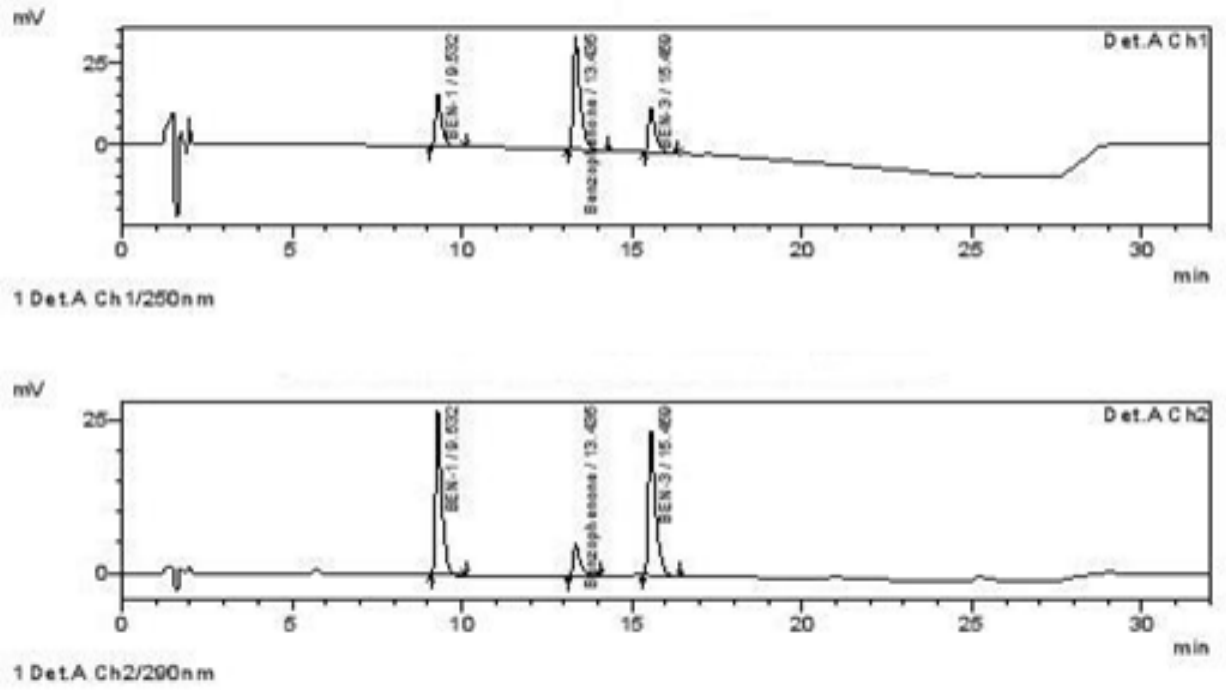

Figure 7: Chromatogram Showing Injection-1 of Standard impurities.

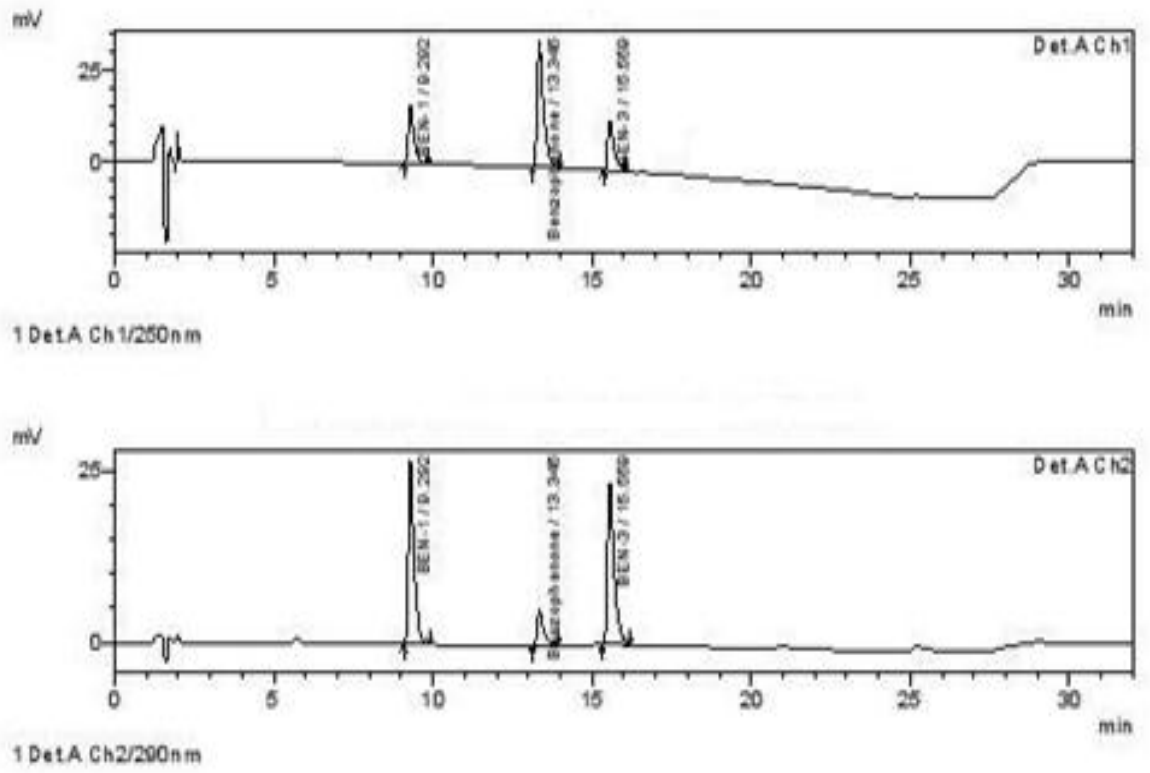

Figure 8: Chromatogram Showing Injection-2 of Standard impurities.

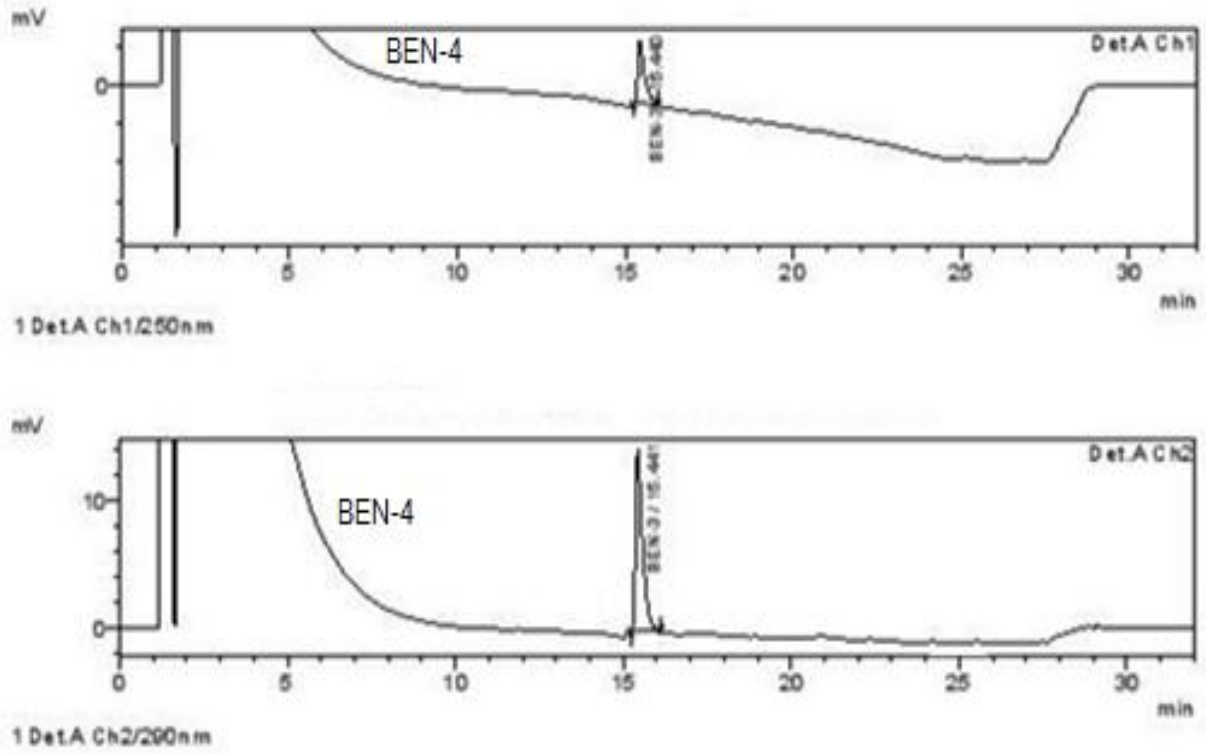

Figure 9 Chromatogram Showing Injection-1 of Sample.

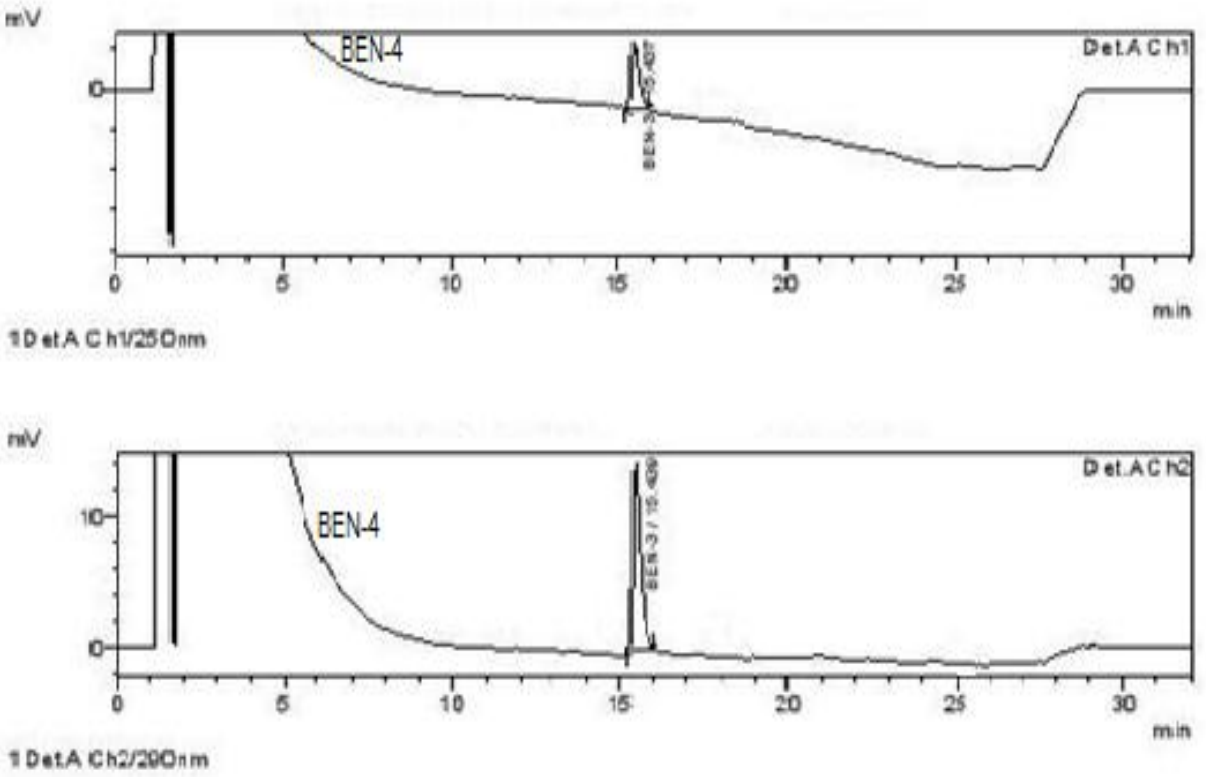

Figure 10 Chromatogram Showing Injection-2 of Sample.

Only Ben-3 was detected in the sample which can be seen in Fig, 9 and 10 its content can be calculated as below.

Ben-3 calculation

Table 1

S.No	Injection Number	Name	Area
1	1	Std.Inj No:1	336858
2	2	Std.Inj No:2	332798
		Mean	334828
		Std.Deviation	2870.85
		% RSD	0.85741

Table 2

S.No	Injection Number	Name	Area
1	1	Sample.Inj No:1	205691
2	2	Sample.Inj No:2	202293
		Mean	203992
		Std.Deviation	2402.75
		% RSD	1.17786

Table 3

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

$$\text{Ben-3 (ppm)} = \frac{\text{Avg area of Ben-3 in sample} \times \text{stdconc} \times 1000000}{\text{Avg area of Ben-3 in standard} \times \text{sample conc}} = 619 \text{ ppm}$$

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 analysis of sample section. This sample solution was spiked with standard 200ppm of Ben-1, Benzophenone, and Ben-3 and 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. 11 and 12.

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

$$A \text{ (content in ppm)} = \frac{\text{Average area of spiked sample} \times \text{stdconc} \times 1000000}{\text{Average area of standard} \times \text{sample conc}}$$

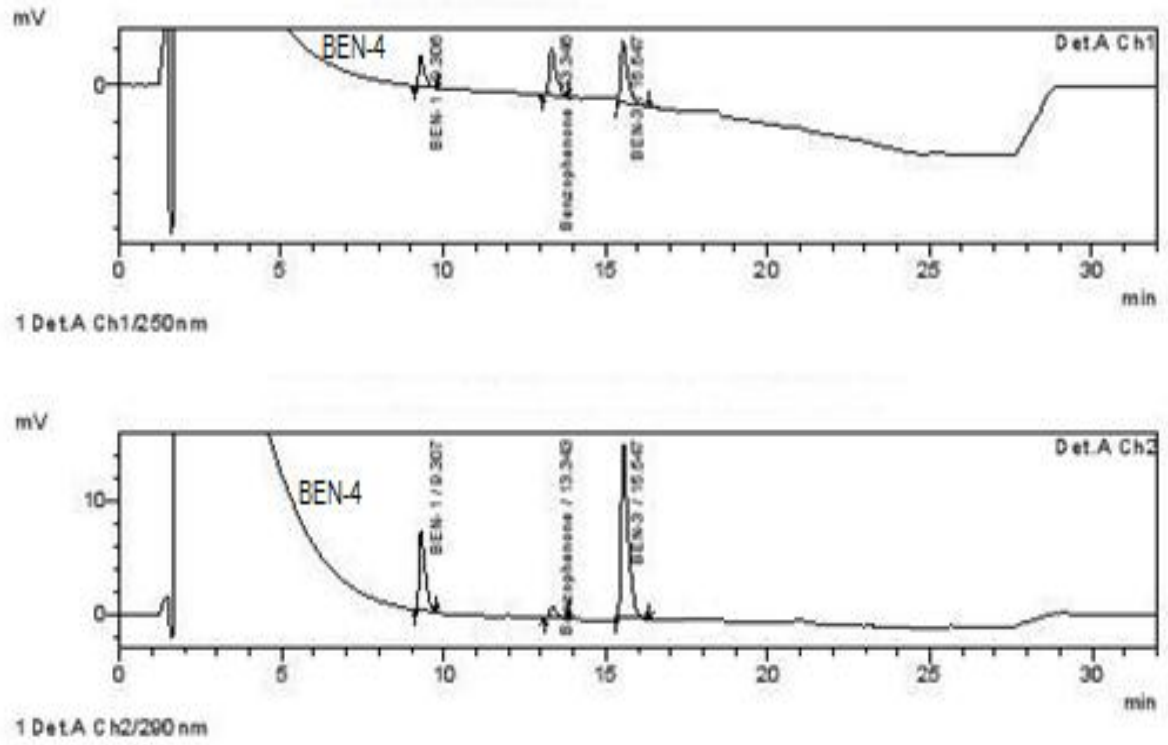

Fig 11: Chromatogram Showing Injection-1 of Spiked Sample.

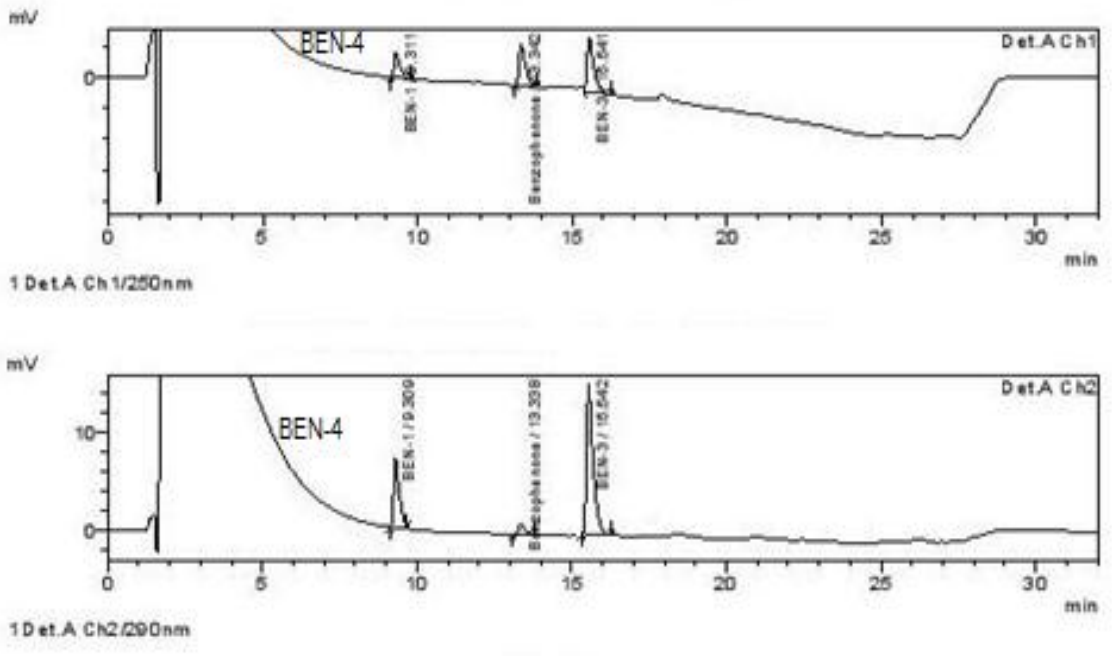

Fig 12 Chromatogram Showing Injection-2 of Spiked Sample.

BEN-3 Content in Spiked Sample

Table 7

S.No	Injection Number	Name	Area
1	1	Std.Inj No:1	336858
2	2	Std.Inj No:2	332798
		Mean	334828
		Std.Deviation	2870.85
		% RSD	0.85741

Table 8

S.No	Injection Number	Name	Area
1	1	Sample.Inj No:1	269274
2	2	Sample.Inj No:2	268693
		Mean	268983.5
		Std.Deviation	410.829
		% RSD	0.152

Table 9

			Dilution(ml)	Ml	ml
Std.Wt	Mg	25.47	50	1	100
Sam.Wt	Mg	125.35	25		

$$\text{Ben-3 (ppm)} = \frac{\text{Avg area of Ben-3 in sample} \times \text{stdconc} \times 1000000}{\text{Avg area of Ben-3 in standard} \times \text{sample conc}} = 816.16 \text{ ppm}$$

Benzophenone Content in Spiked Sample

Table 10

S.No	Injection Number	Name	Area
1	1	Std.Inj No:1	482670
2	2	Std.Inj No:2	478034
		Mean	480352
		Std.Deviation	3278.147
		% RSD	0.68244

Table 11

S.No	Injection Number	Name	Area
1	1	Sample.Inj No:1	93898
2	2	Sample.Inj No:2	93730
		Mean	93814
		Std.Deviation	118.793
		% RSD	0.12662

Table 12

			Dilution(ml)	Ml	Ml
Std.Wt	Mg	25.41	50	1	100
Sam.Wt	Mg	125.35	25		

$$\text{Benzophenone(ppm)} = \frac{\text{Avg area of Benzophenone in sample} \times \text{stdconc} \times 1000000}{\text{Avg area of Benzophenone in standard} \times \text{sample conc}}$$

= 198.42 ppm

Ben-1 Content in Spiked Sample

Table 16

S.No	Injection Number	Name	Area
1	1	Std.Inj No:1	337260
2	2	Std.Inj No:2	335224
		Mean	336242
		Std.Deviation	1439.67
		% RSD	0.42816

Table 17

S.No	Injection Number	Name	Area
1	1	Sample.Inj No:1	65198
2	2	Sample.Inj No:2	64974
		Mean	65086
		Std.Deviation	158.3
		% RSD	0.234

Table 18

			Dilution(ml)	ml	Ml
Std.Wt	Mg	25.47	50	1	100
Sam.Wt	Mg	125.35	25		

$$\text{Ben-1 content(ppm)} = \frac{\text{Avg area of Ben-1 in sample} \times \text{stdconc} \times 1000000}{\text{Avg area of Ben-1 in standard} \times \text{sample conc}}$$

= 196.65 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, and Ben-3 in sulisobenzone and validate it for accuracy and applying the same for its estimation in Sulisobenzone sample.

In Sulisobenzone sample only Ben-3 was detected and its content was found to be 619.06 ppm. The Recovery results obtained for this method were promising. Hence the developed method can be adopted for determination of Benzophenone, Ben-1, Ben-3 in sulisobenzone in quality control laboratories.

ACKNOWLEDGEMENTS

Authors would like to thank Anurag Group of Institutions. for providing facilities and materials to carry the research work. Authors would also like to thank MrAkifulhaqueAsst Professor Anurag Group of Institutions for his valuable suggestions

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