Journal of Applied Pharmaceutical Science Vol. 4 (09), pp. 052-055, September, 2014
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2014.40909
ISSN 2231-3354 

# Development of colorimetric method for the quantification of methyl salicylate in bulk and formulations

Sajin Kattuvilakam Abbas[1], Suseem Sundaram Rengitham[2]

[1] Department of Pharmaceutical chemistry, Karavali College of Pharmacy, Mangalore, Karnataka, India.
[2] Pharmaceutical Chemistry Division, School of Advanced Sciences, Vit University, Vellore-632014, Tamil Nadu, India.

**ARTICLE INFO**

*Article history:*
Received on: 11/07/2014
Revised on: 09/08/2014
Accepted on: 21/08/2014
Available online: 27/09/2014

***Key words:***
Methyl salicylate, Ferric chloride, colorimetry.

**ABSTRACT**

Methyl salicylate is chemically methyl-O-hydroxybenzoate and act as an active ingredient in many topical analgesic preparations. It posses good counter irritant activity. In this present study, a colorimetric method is developed for the quantitative estimation of methyl salicylate, both in the bulk form and in the formulations. A dark bluish – purple colour is formed between methyl salicylate and ferric chloride reagent. The intensity of the colour is directly related to the concentration of methyl salicylate present. The developed method was simple and gave accurate and precise results. The developed colour had its maximum value of absorbance at 537 nm when scanned in the visible region. The absorbance of the coloured system was measured at 537 nm. Beer's law was obeyed in the range of 12 to 72 µg/mL. The equation for the straight line was found to be, y = 0.0075 x + 0.002 with correlation coefficient value of 0.996. The developed method was applied to semi – solid formulations for the quantitative estimation of methyl salicylate. The percentage of recovery was between 99.62 % in the formulation. The method proved to be a convenient analytical tool for the quantity determination of methyl salicylate.

## INTRODUCTION

Methyl salicylate, also known as Oil of winter green, is chemically methyl-O-hydroxy benzoate (Fig. 1) and act as an active ingredient in many topical analgesic preparations. It is produced naturally and is found in variety of plants especially belonging to the species like Gaultheria, Betula etc. It can also be synthetically produced (Zanger and Mckee, 1988; Hartel and Hanna, 2009), and is used in foods and beverages as a fragrance agent in very minute quantities. In medicine it finds use as an agent for external application possessing pain relieving and counter irritant activities (Ragan *et al.*, 2004).

It is a subject of monograph in British pharmacopeia (British Pharmacopeia, 2009). Quantitative estimation of the active ingredient is of utmost importance when a drug is developed to a formulation. Also this is required to find the percentage of purity to check the standard of the drug in bulk. The literature survey conducted on the quantitative estimations of methyl salicylate revealed that there are so many instrumental chromatographic methods available.

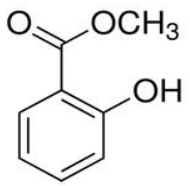


**Fig. 1:** Methyl Salicylate.

HPLC and GC methods are reported so far for the quantification of methyl salicylate (Mills PC and Cross SE,2007; Cross SE *et al.*, 1998; Krzek *et al.*, 2003; Sapio *et al.*, 2006). There is also one Ultraviolet spectrophotometric method reported (Dorwal, 2012). The British Pharmacopeia suggests the acidometry followed by saponification for the assay of the drug (British Pharmacopeia, 2009). There are no colorimetric estimations reported for the compound so far. Hence, the attempt to develop an analytical method based on the general reaction of the phenolic group present in the compound is performed in this present study.

* *Corresponding Author*
*Suseem Sundaram Rengitham, , Pharmaceutical Chemistry Division, School of Advanced Sciences, VIT University, Vellore-632014, Tamil Nadu, India. Email: srsuseem@vit.ac.in*

The characteristic reaction of Phenols reacting with Ferric chloride reagent produced a bluish violet colour (Wesp and Brode, 1934) is the basis of the present study. Therefore an attempt to develop a simple, accurate and precise method which enables the quantification in an easy manner is performed.

## EXPERIMENTAL METHODS

### Instruments

The instruments needed for the study included the spectrophotometer and the analytical balance. Schimadzu UV-1601 and Schimadzu AW 220, respectively were the spectrophotometer and the analytical balance used.

### Glasswares

All the glassware's required for the trials were made of borosilicate glass and were the product of the renowned manufacturer Borosil.

### Chemicals and Reagents

The reagents for the study included Ferric chloride, Methyl salicylate and ethanol. All the reagents were procured from S D scientifics, Mangalore, India and were of analytical grade. The marketed formulation used for the study was Volitra gel, a product of Ranbaxy, containing 10% w/w quantity of the compound .

### Preparation of Standard stock solution

The standard solution of the compound was prepared by taking Methyl salicylate and dissolving in ethanol to a final concentration of 120 µg / mL. This was followed by taking different aliquots from the stock solution and adding 1 mL of freshly prepared solution of 1 % w/v ethanolic ferric chloride. All the aliquots were diluted to a concentration range of 12 to 72 µg /mL (Mendhem *et al.*, 2008)

### Preparation of sample solution

The formulation equivalent to 10 mg of the compound was weighed and transferred to a volumetric flask of 100 mL capacity. About 50 mL of ethanol was added and shaken well for 2 minutes. The volume was made upto the mark with more quantity of ethanol. This solution was filtered through What-man Grade No 1 filter paper and filtrate was collected in another volumetric flask. About 2 mL of this solution was transferred to a 10 mL volumetric flask and 1 mL of 1 % w/w ethanolic ferric chloride reagent was added. The volume was made upto the mark with ethanol.

### Preparation of the reagent

About 1 gram of Ferric chloride reagent was weighed accurately and transferred to a volumetric flask of 100 mL capacity. To the above reagent, 50 mL of ethanol was added and shaken well for the reagent to get dissolved. Then the volume was then made up using ethanol.

## METHODOLOGY

### Determination of maximum absorbance value

The coloured system of standard drug was scanned in the region of 380 -760 nm for the determination of wavelength of maximum absorbance. The maximum absorbance was noted at 537 nm.

### Linearity

The linear relationship of absorbance against concentration was studied. The absorbances of standard solutions ranging from 12 to 72 µg / mL were measured at 537 nm. The results for the trials are tabulated in **Table 1.**

**Table 1:** Linearity data of Absorbance against Concentration.

| Concentration (µg /mL ) | absorbance |
|---|---|
| 12 | 0.093 |
| 24 | 0.172 |
| 36 | 0.273 |
| 48 | 0.361 |
| 60 | 0.440 |
| 72 | 0.540 |

From the results it proved that there exists a linear relationship between the absorbance and the concentration in the above said range of concentrations. Hence this range was selected as the linear range obeying the Beer Lambert law for the determination. The calibration curve is given in Fig. 2.

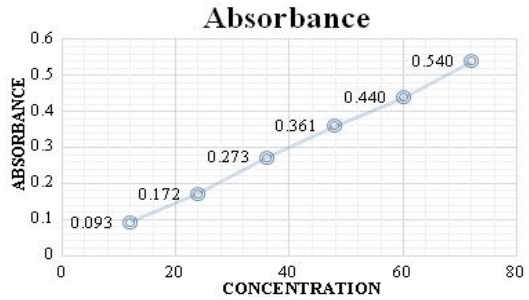


**Fig. 2:** Plot showing the absorbance against concentration.

### Application of the method for quantification of the drug

The absorbance of the sample solution was measured keeping all the parameters same as that for the standard. The value obtained was compared with that of the values in the calibration curve and concentration of the drug in the diluted sample was found out. The concentration in the undiluted sample was calculated next. The quantity was found to be equal to that of the label claim.

### Study of matrix effects in trials

Six volumetric flasks of 10 mL capacity were taken. To all of them was added 1 mL of the ultimately diluted sample solution of uniform concentration (100 µg). About 1 mL of Standard solutions having concentrations of 12, 24, 36, 48, 60 µg / mL were added to each flask and labeled. Of the total six flasks taken, one was retained without the addition of the standard. To all

the six flasks was added 1 mL of freshly prepared solution of 1% w/v of ethanolic ferric chloride reagent.

All the flasks were made up to the volume using ethanol. The absorbance of the colour developed was measured at 537 nm using ethanol as blank. The results obtained are tabulated in the Table 2. The values of absorbance were then plotted against the concentration of the standard added, the straight line obtained was extrapolated and the quantity of drug in the sample was calculated. This step helped to overcome any possible matrix effect that would affect the estimation leading to error in results. A curve representing this study is shown in Fig. 3.

**Table 2:** Absorbance for the samples after the addition of standard.

| Sl no | Concentration of standard added (µg /mL) | Absorbance |
|---|---|---|
| 1 | 0 | 0.069 |
| 2 | 12 | 0.077 |
| 3 | 24 | 0.085 |
| 4 | 36 | 0.094 |
| 5 | 48 | 0.102 |
| 6 | 60 | 0.110 |

**Table 3:** Results for determination of accuracy ( recovery study method ).

| Quantity present In the sample (µg) | Level of addition ( % ) | Quantity of standard Added (µg) | Quantity Recovered (mcg) (n = 3) | % recovery Mean ± SD | Average |
|---|---|---|---|---|---|
| 20 | 80 | 16 | 35.67 | 99.08 | |
| 20 | 100 | 20 | 39.80 | 99.5 | 99.62% |
| 20 | 120 | 24 | 44.13 | 100.29 | |

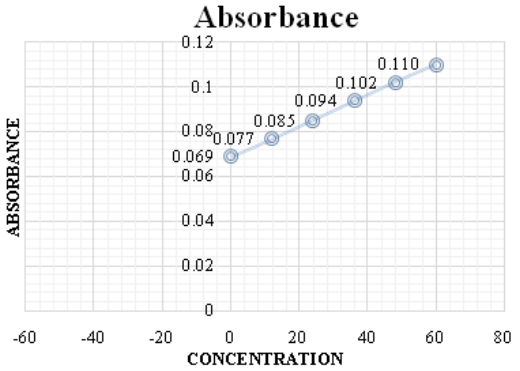


**Fig. 3:** Plot representing the method of standard addition to study the matrix effect.

**Sensitivity**

Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated to express the sensitivity of the developed method. LOD and LOQ were calculated using the expressions, LOD = 3.3 x SD/S, and LOQ = 10 x SD/S , where SD stands for the standard deviation and S for the slope of the line in the calibration curve. The LOD and LOQ were found to be respectively 0.0735 and 0.223 µg /mL.

**Accuracy**

A multiple level recovery study was done to check the accuracy of the method. Standard additions were done at three levels viz 80 %, 100 % and 120 %. Standard drug was added to a fixed amount of the pre analyzed sample and the amounts of the drug were quantified by the proposed method. The result for the recovery is given in Table 3.

**Precision**

The method proved to be precise with respect to both the types viz repeatability and reproducibility. The trials were repeated six times the same day for which the values were in the range of concordance. The trials were also done to check the inter day precision which gave reliable results. The details of the results of the precision are given in **Table 4.**

**Table 4:** Results for determination of Precision.

| Serial Number | Percentage of label claim | |
|---|---|---|
| | Repeatability | Reproducibility |
| 1 | 98.64 | 99.66 |
| 2 | 99.14 | 98.54 |
| 3 | 99.36 | 99.62 |
| 4 | 99.68 | 97.24 |
| 5 | 99.72 | 99.42 |
| 6 | 99.63 | 98.66 |
| Coeffecient of variation ( % ) | 0.01 | 0.04 |

## RESULTS AND DISCUSSION

The colorimetric method was optimized with an objective to develop a simple, accurate and stable assay method for the drug Methyl salicylate in bulk and in formulations. The sample of the drug when treated with ethanolic Ferric chloride reagent, produced a bluish – purple which had its maximum value of absorbance at a wavelength of 537 nm. The dilution of the prepared aliquots was done using pure ethanol owing to the reason of miscibility of drug with ethanol. The determination of the absorbance of the colour produced in various aliquots was done at a wavelength of 537 nm.

The linearity was assessed by plotting absorbance against the concentration, shown in **Table 1**, and found that the linearity existed in the range of 12 to 72 µg /mL, with the correlation coefficients 0.996. The accuracy of the proposed work was checked by performing recovery studies and the percentage recovery was observed to be within the range of acceptance. The average percentage of recovery was found to be 99.62%. The coefficient of variation for the repeatability and reproducibility precisions were less than 1%.

The limit of Detection and Limit of Quantification for the drug by the proposed method were 0.0735 and 0.223 µg / mL respectively.

## CONCLUSION

The proposed study was a success in establishing a new and simple colorimetric estimation of Methyl salicylate in bulk and in semi-solid formulations. The method has been validated and was found to be simple, rapid, sensitive, accurate and precise one. Moreover the requirement of easily available reagents that are inexpensive and safe to handle makes the method analyst friendly

one and economic. The proposed method can be used for the quantification of Methyl salicylate in bulk and in formulations.

## REFERENCES

Zanger M, Mckee JR. The synthesis of Methyl Salicylate: Amine Diazotisation, J Chem.Educ,1988,65(12),p 1106. DOI: 10.1021/ed065p1106.

Hartel AM , Hanna JM. Preparation of oil of Wintergreen from commercial Aspirin tablets. A microscale experiment highlighting Acyl substitutions, J.Chem. Educ, 2009, 86(4) p 475. DOI: 10.1021/ed086p475.

Ragan BG, Nelson AJ, Foreman JH, Bell GW, Iwamoto GA, Effects of a menthol based analgesic balm on pressor responses evoked from muscle afferents in cats, Am J Vet Res 2004,65:1204 – 1210. DOI: 10.2460/ajvr.2004.65.1204

British Pharmacopeia, The British Pharmacopeia Commission, London, 2009,3859

Mills PC , Cross SE. Regional differences in the *in-vitro* penetration of methyl salicylate through equine skin. Veter J. 2007;173:57-61. PMID: 16246600

Cross SE, Anderson C and Roberts MS. Topical penetration of commercial salicylate esters and salts using human isolated skin and clinical microdialysis studies. Br J clin Pharmacol. 1998;46:29-35. doi/10.1046/j.1365-2125.1998.00045.x/pdf.

Krzek J, Czekaj JS and Rzeszutko W. Validation of a method for simultaneous estimation of menthol and methyl salicylate in pharmaceuticals by capillary gas chromatography with cool on column injection. Acta Pol Pharm Drug Res. 2003;60(5):343-49.

Sapio JP, Sethachutkul K and Moody JE. Simultaneous GLC determination of methyl salicylate and menthol in a topical analgesic formulation. J Pharm Sci. 2006;68(4):506-8. DOI: 10.1002/jps.2600680431

Dharwal Dorwal, Development of UV spectrophotometric method for determination of methyl salicylate in bulk and semi solid formulation, Int. Journal of research in Pharma and Biomedical sciences, Vol. 3 (3), Jul- sep 2012 : 983 – 986.

British Pharmacopeia, The British Pharmacopeia Commission, London, 2009,3860.

Wesp EF, Brode WR, The absorption spectra of Ferric compounds.1.The Ferric chloride – Phenol Reaction, J Am Chem. Soc, 1934, 56( 5), pp: 1037 – 1042. DOI: 10.1021/ja01320a009

Mendham J, Denney R C, Barnes J D and Thomas M J K,2008, Colorimetric determinations: general procedure. In:Vogel's Textbook of Quantitative Chemical Analysis., Dorling Kindersley (India) Pvt. Ltd 683-85.

**How to cite this article:**

Sajin Kattuvilakam Abbas, Suseem Sundaram Rengitham. Development of Colorimetric Method for the Quantification of Methyl Salicylate in Bulk and Formulations. J App Pharm Sci, 2014; 4 (09): 052-055.