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Original Article

Spectrophotometric determination of ichthammol in topical formulations

Gamze Ergin Kızılçay[1]

[1]İstanbul University, Faculty of Pharmacy, Department of Analytical Chemistry, İstanbul, Türkiye

ABSTRACT

Background and Aims: This study developed a new, simple, and rapid spectrophotometric method for determining ichthammol as a local antiseptic in topical formulations. The developed spectrophotometric method for determining ichthammol was applied to topical formulations.

Methods: The linearity range of the ichthammol was 5.0-50.0 µg/mL at a wavelength of 235 nm. Ethanol:water (50:50; v/v) was used as the solvent. The method was validated using the limit of quantification, limit of detection, linearity, selectivity, robustness, recovery, precision, stability, and accuracy of the method using the International Conference on Harmonisation guidelines for validation of analytical procedures: text and methodology Q2 (R1).

Results: The limits of detection and quantification were calculated to be 0.044 µg/mL and 0.148 µg/mL, respectively. Intra-day and inter-day relative standard deviation values were calculated to be less than 1.024 %. The assay recovery and precision of ichthammol from topical formulations at 5.0, 20.0, and 50.0 µg/mL were evaluated. The mean recoveries for ichthammol in the topical formulation were calculated at 97.944-99.860%. It was determined that the ichthammol sample solution was stable for 24 h at 4.0°C. The validated method was applied to the cream formulation, and the amount of ichthammol in the cream was determined to be 97.442%.

Conclusion: The validated method was successfully applied to determine ichthammol in topical formulations. The proposed method is reproducible and reliable and can be safely used for routine analysis.

Keywords: Ichthammol, spectrophotometric method, topical formulation, validation

INTRODUCTION

Ichthammol, also known as dark sulphonated shale oil, is a black, oily compound with a characteristic odour. From elemental analysis, the composition of ichthammol was calculated to be $C_{28}H_{36}S_5O_6(NH_4)_2$ (Baumann & Schotten, 1883). It is an ammonium salt of dark sulphonated shale oil (bituminous schists) produced via distillation, followed by sulfonation and neutralisation (Österreichischen Ichthyol Gesellschaft, 1884). An active substance belonging to the class of antiseptics and disinfectants, it is used topically (Boyd, 2010). Ichthammol can be used as a local antiseptic or in the treatment of inflammatory diseases of the skin (such as eczema, abscess, and boil) due to its irritant properties and as an aid in the treatment of chronic skin diseases together with other antiseptics (Shi, Hsiao, Lowes, & Hamzavi, 2021).

Although the first use of ichthammol for treating dermatological diseases was reported by Paul Unna in 1882 (Unna, 1882), scientific data regarding its effectiveness for treating dermatological diseases are quite limited. The majority of research on the topic is from Europe (Boyd, 2010). Currently, products containing ichthammol are used as a local antiseptic for eczema, furunculosis, psoriasis, and acne. Due to its irritating effect on the skin, it is used together with other antiseptics to treat boil-like inflammations and skin diseases such as erysipelas (snapworm) and lupus erythematosus (erythematous skin tuberculosis). It is also popularly advocated for use as a "drawing ointment" for use in relieving glass or wood splinters, spider bites, arthropod attacks, and abscesses (Boyd, 2010).

Ichthammol is available over-the-counter in many forms but is most commonly included in pharmaceutical formulations along with zinc, paraffin, and beeswax, usually in concentrations of 5-20%. In the case of boils, topical application is thought to accelerate their “marking” by more easily expelling their contents (i.e., “drawing”). There are no absolute contraindications to the use of ichthammol, but because it is believed to have anti-inflammatory, antibacterial, and antimycotic qualities, it is accessible as an active ingredient in over-the-

Corresponding Author: Gamze Ergin Kızılçay **E-mail:** gamze.erginkizilcay@istanbul.edu.tr
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counter treatments for the treatment of conditions like offensive odour and skin irritation. (DrugBank, 2006).

Since topical ointments are non-prescription and have been used by the public since antiquity, there is no reliable method to measure the amount of ichthammol present in them. Despite this, topical ointments are frequently used in dermatology. Existing methods in pharmacopoeias (USP Pharmacopoeia, 2012) and the literature (Perevozchikova & Savelyeva, 1981; Kalde Ya, 1976; Saveleva & Kudymov, 1963; Suprun, 1961) are indirect methods based on the determination of molecules such as iodine, sulphur, and ammonia.

In this study, we aimed to develop a fast, easy, and inexpensive spectrophotometric method for the analysis of this compound from pharmaceutical preparations in ointment formulations. The developed method was validated according to the International Conference on Harmonisation (ICH) Harmonised Tripartite Guideline Validation of analytical procedures: text and methodology Q2 (R1) (ICH Q2 (R1), 2005) rules and was applied to analyse topical pharmaceutical preparations containing commercial ingredients on the market.

MATERIAL AND METHODS

Chemicals

Ichthammol was purchased from Österreichischen Ichthyol Gesellschaft m.b. H. & Co. KG. Ultrapure water from an Elga-branded water system was used in the research laboratory. ACS-grade ethanol was obtained from Merck (Germany). A pharmaceutical topical formulation was purchased fom a pharmacy in Turkey.

Solutions

The stock solution of ichthammol (1.0 mg/mL) was prepared in ethanol-water (50:50, v/v), and it was diluted using ethanol-water (50:50, v/v) to obtain a second stock solution of 100.0 µg/mL. Volumes of 0.5 mL were taken from the second stock solution, and 0.1-0.5 mL were taken from the stock solution diluted to 10.0 mL using the ethanol-water to prepare six practical ichthammol solutions at different concentrations (5-50 µg/mL). The linearity range of ichthammol was 5.0, 10.0, 20.0, 30.0, 40.0, and 50.0 µg/mL.

Instrumentation

A UV-160 A spectrophotometer of the Shimadzu brand (Kyoto, Japan) was used in this study. Measurements were carried out at a wavelength of 235 nm using 1-cm quartz cells.

Preparation of the sample and placebo solutions

The sample and placebo solutions were prepared in ethanol:water (50:50, v/v). The topical formulation and placebo were weighed at 50.0 mg into a 250.0 mL volumetric flask and dissolved in ethanol-water (50:50, v/v). The sample solution was stirred in a vortex mixer for 2.0 min and then placed in an ultrasonic bath at 40.0 °C for 30.0 min. It was completed to its volume with ethanol-water. The solution was brought to room temperature by keeping it under tap for 2.0 min and completed to its volume with ethanol-water (50:50; v/v) solution. Vortexing was performed for 2.0 min. The absorbance of the sample was measured at 235 nm against a blank (ethanol:water (50:50; v/v), which was selected as the maximum wavelength. The amount of substance in the topical formulation was calculated by substituting the resulting absorbance into the calibration equation.

Method development and validation

During method development, the measurement wavelength was first determined. Then, measurements were taken at different solvent ratios, and the best solvent ratio was determined. For sample preparation conditions, the ultrasonic bath holding time and temperature were tested. The developed method was validated using the ICH Guidelines Q2 (R1) as a reference (ICH Q2 (R1), 2005).

Taking into consideration the concentration of ichthammol present in the preparation containing solution, the linearity of ichthammol was investigated in the range of 5.0-50.0 µg/mL (n=3). The limits of detection (LOD) and limits of quantitation (LOQ) were calculated using the formula LOD or LOQ = κSDa/b, where the value of K is 3 and 10 for LOD and LOQ, respectively.

By selecting the three calibration curve concentrations, absolute recoveries were evaluated by adding placebo to concentrations in three selected calibration curves. A placebo solution was prepared in a similar manner to the sample solution. Three different concentrations of standard ichthammol solutions (5.0, 20.0, and 50.0 µg/mL (n=3), were filled to their volume with the placebo solution, and these mixtures were analysed using the proposed method.

Precision studies on the method were examined for inter-day and intra-day precision. Therefore, separate standard solutions were prepared at concentrations of 5.0, 20.0, and 50.0 µg/mL (n=3). The standards from the same day and different days were analysed and assessed by calculating the relative standard deviation (RSD) percentages of the field values.

The stability of ichthammol in the topical formulation solution was studied at the end of the 12th and 24th hours while the samples were kept under 4.0°C conditions. Stability was assessed by comparing the initial results with the results obtained at the conclusion of the analyses at the end of the 12th and 24th hours. The robustness parameter was evaluated by varying the wavelength and solvent ratio. Three replicates were determined using a standard solution for each robustness parameter. The standard solution was initially prepared in ethanol:water

(50:50, v/v). Subsequently, measurements were conducted after preparing the standard solutions using solvent ratios of 48:52, v/v ethanol to 52:48, v/v water. A similar approach was adopted for wavelength, with initial measurements made at the method-specified wavelength of 235 nm. The standard solution was measured by adjusting the wavelength from 233 to 237 nm. The measured values obtained were analysed for the RSD value, which was selected as the system suitability parameter.

RESULTS AND DISCUSSION

During the method development studies, the best spectrophotometric measurement results were obtained using the solution ratio of ethanol-water (50:50, v/v) (Figure 1). The highest absorbance values were obtained at 40.0 °C (Figure 2) and 30.0 min (Figure 3) in the ultrasonic bath for sample preparation. The measurement wavelength was 235 nm. Validation studies under these conditions were conducted as follows.

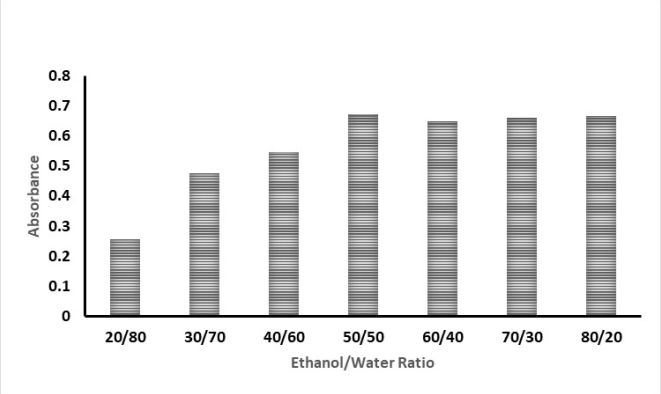

Figure 1. Effect of solvent ratio on the absorbance of the ichthammol solubility

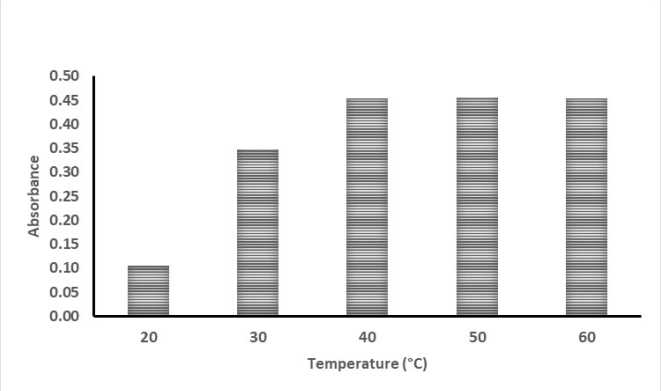

Figure 2. Effect of temperature on absorbance in sample preparation

Selectivity

To assess method selectivity, absorbance was measured in the solvent, ichthammol standard solution, topical formulation, and

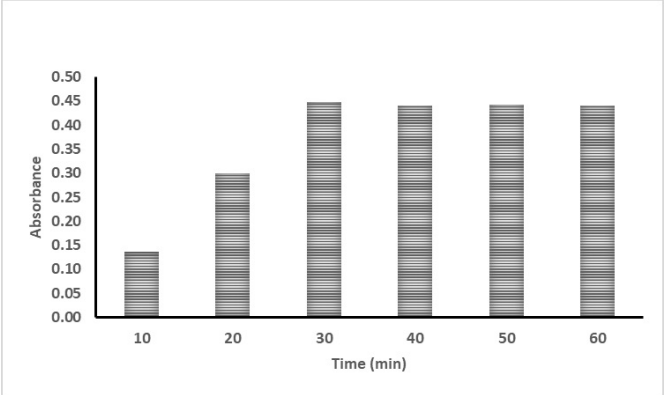

Figure 3. Effect of time on absorbance in sample preparation

placebo solutions. During the ichthammol spectrum, no absorbance was observed due to the solvent or placebo, as depicted in Figure 4.

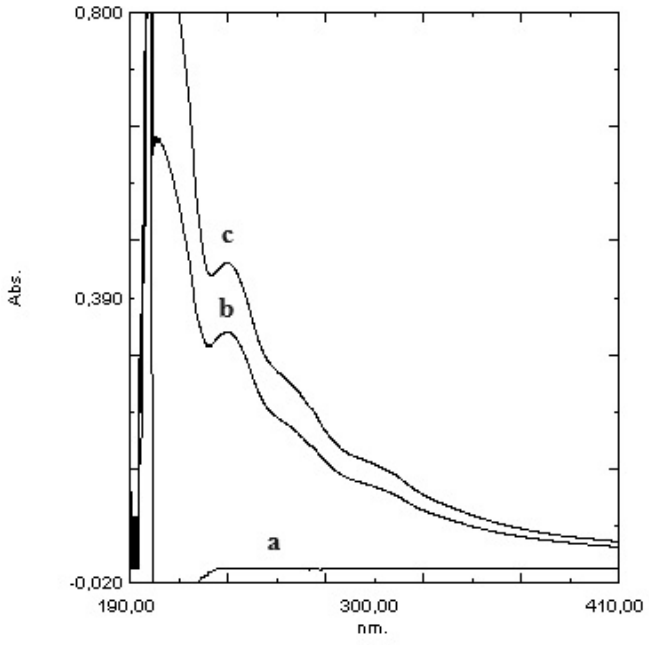

Figure 4. The absorption spectrum of a) the placebo solution, b) the standard ichthammol solution (20.0 µg/mL), c) the cream formulation (10%)

Linearity and sensitivity

Considering the analysed ichthammol formulation, the linearity of the proposed method was studied in the concentration range of 5.0-50.0 µg/mL. The average regression formula can be expressed as A = 0.0191 C + 0.0633 (R^2=0.9992) with C representing the concentration of ichthammol (µg/mL) and A represents the absorbance. The linearity results obtained by the proposed method are displayed in Table 1. In accordance with

the study parameters, LOD and LOQ were calculated at 0.044 µg/mL and 0.148 µg/mL, respectively.

Table 1. Linearity results obtained by the developed method

Parameter	Ichthammol
Linearity range (µg/mL)	5.0-50.0
Regression equation	A = 0.0191 C + 0.0633
Slope ± SD	0.0191± 4.71x 10^{-5}
Intercept ± SD	0.0633± 2.83 x10^{-4}
Mean correlation coefficient, R^2	0.9992
LOD[a] (µg/mL)	0.044
LOQ[b] (µg/mL)	0.148

[a] Limit of Detection; [b] Limit of Quantitation

Recovery

As shown in Table 2, the absolute recovery values of ichthammol in the topical formulations were between 97.944 and 99.860%. The average ichthammol recovery was 98.806%.

Table 2. Recovery results for the assay of ichthammol (n=3)

Concentration (µg/mL)		Recovery (%)	RSD[b] (%)
Added	**Found (mean ± SD[a])**		
5.0	4.993±0.030	99.860	0.605
20.0	19.723±0.091	98.613	0.460
50.0	48.972±0.132	97.944	0.269

[a] Standard deviation; [b] Relative standard deviation

Precision

Precision assessments were performed for both intra-day and inter-day repeatability, as previously described. The RSD% for intra-day repeatability ranged from 0.394% to 1.024%, and for inter-day repeatability, it ranged from 0.280% to 0.593%. Table 3 lists the precision values of the method. These results are in accordance with the statement that the RSD% should be less than 2.0%.

Stability

By comparing the preliminary findings with those obtained at the end of the studies after 12 and 24 h, the developed method

Table 3. Intra-day & inter-day precision and accuracy of ichthammol (n=3)

Concentration (µg/mL)			
Added	**Found (mean ± SD[a])**	**RSD[b] (%)**	**RME[c] (%)**
Intra-day			
5.0	5.115 ± 0.052	1.024	2.304
20.0	20.316 ± 0.080	0.394	1.579
50.0	49.827 ± 0.262	0.525	-0.346
Inter-day			
5.0	5.098 ± 0.030	0.593	1.955
20.0	20.211 ± 0.080	0.396	1.056
50.0	49.513 ± 0.138	0.280	-0.974

[a] Standard deviation; [b] Relative standard deviation; [c] Relative mean error

stability under specified conditions for the ichthammol solution was evaluated. Upon analysing the obtained values, it was evident that the variations ranged from 100.59% to 101.04% (Table 4). These results show that there was no noticeable change in the absorbance.

Table 4. Stability results of ichthammol obtained by the proposed method (n=3)

Time (hour)	Concentration (µg/mL) (mean±SD)	RSD (%)	Variation (%)
0	20.281±0.030	0.149	100.00
12	20.525±0.030	0.147	101.04
24	20.421±0.030	0.148	100.59

Robustness

To evaluate the robustness of the method, the results obtained by changing the solvent ratio and wavelength were evaluated. The RSD% value determined using the proposed method was 0.128 at a solvent ratio of 50:50, v/v, and wavelength of 235 nm. During the robustness assessment of the proposed method, adjustments were made to the solvent ratio and wavelength as previously outlined. The resulting RSD% values were within the range of 0.126-0.129 and 0.129-0.131, respectively. Additionally, the variation% was 99.630-101.554 for solvent ratio and 98.881-99.030 for wavelength changes. It was determined that the proposed method was not affected by minor changes.

The developed and validated spectrophotometric method is

simple to prepare, selective, reproducible, rapid, and reliable, enabling the analysis of ichthammol from topical formulations.

Determination of Ichthammol in Topical Formulation

The percentage of ichthammol in the cream formulation, as determined using the proposed method, was calculated to range from 9.536% to 9.924% (Table 5). This result proves the successful application of the method in analysing ichthammol within the cream preparation.

Table 5. Determination of ichthammol in topical formulation (n=6)

n	g / 20 g	%
1	1.962	9.812
2	1.985	9.924
3	1.952	9.758
4	1.931	9.655
5	1.907	9.536
6	1.956	9.780
Mean	1.949	
SD[a]	0.027	
RSD[b]	1.373	

[a] Standard deviation; [b] Relative standard deviation

CONCLUSION

In this study, a fast, easy, and inexpensive spectrophotometric method was developed for the analysis of ichthammol, which is widely used among the public for pharmaceutical preparations in ointment form. The developed method was validated according to the ICH guidelines and applied to the analysis of topical pharmaceutical preparations commercially available on the market.

The developed method was carried out directly via spectrophotometric measurement for the analysis of ichthyol and is the first direct method ever published. In contrast, previously published methods were based on the indirect analysis of substances such as sulphur and ammonia in the content of ichthammol (Perevozchikova & Savelyeva, 1981; Kalde Ya, 1976; Saveleva & Kudymov, 1963; Suprun, 1961). Additionally, the method's sample preparation step is straightforward for topical formulations because it only requires 30.0 min of ultrasonic bath heating at 40.0°C. Method validation studies have demonstrated that this approach is appropriate for highly accurate, precise, and robust quantification of drugs from topical formulations.

As a result, the proposed method allows the analysis of ichthammol in topical pharmaceutical preparations to be performed directly, simply, and quickly, with high accuracy, precision, and robustness, and the method can be easily applied to routine analyses.

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ORCID IDs of the authors

Gamze Ergin Kızılçay 0000-0002-1089-7195

REFERENCES

Baumann, E., & Schotten, C. (1883). Über das "Ichthyol"". *Monatshefte für praktische Dermatologie*, 2, 257.

Boyd, A.S. (2010). Ichthammol revisited. *International Journal of Dermatology*, 49(7), 757– 760. https://doi.org/10.1111/j.1365-4632.2010.04551.x

DrugBank Online (2006). Retrieved from https://go.drugbank.com/drugs/DB11341 (Updated at September 28, 2021).

ICH Q2 (R1). (2005). Validation of Analytical Procedures: Text and Methodology, https://database.ich.org/sites/default/files/Q2%28R1%29%20Guideline.pdf. (Accessed October 8, 2023).

Kalde Ya., L. (1976). Determination of sulphate sulfur in ichthyol by the titrimetric method (Russian). *Farmatsiya*, 25(2), 69-71.

Österreichischen Ichthyol Gesellschaft m.b.H. & Co. KG (1884). Retrieved from https://www.ichthyol.de/en/raw-materials-for-corporate-customers/ ichthammol/

Perevozchikova, G.G., & Savelyeva, G.I. (1981). Quantitative determination of potassium iodide in suppositoria with ichthyol. *Farmatsiya*, 30(2), 62-63.

Saveleva, G.I., & Kudymov, G.I. (1963). Qualitative Determination of Thiophene Sodium in Ichthyol. | Kolıchestvennoe Opredelenıe Tıofenovo I Sery V Ikhtıole. *Aptechnoe delo*, 12, 62-65.

Shi, V.Y., Hsiao, J.L., Lowes, M.A., & Hamzavi, I.H. (2021). A Comprehensive Guide to Hidradenitis Suppurativa. Elsevier. https://doi.org/10.1016/C2019-0-04427-5.

Suprun, P.P. (1961). Iodo-chlorometric method for quantitative determination of ichthyol in. *Aptechnoe delo*, 10, 38-42.

The United States Pharmacopeia (USP) 35 (2012). Ichthammol, 3474-3475.

Unna, P.G. (1882). Aphorismen über Schwefeltherapie und Schwefelpräparate. IV. Ichthyol. *Monatsheft Praktische Dermatologie*, 1, 328-333.

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