Research Article

Determination of Dyclonine Hydrochloride by a HPLC Method and Camphor and Menthol by a GC Method in Compound Lotion

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A high performance liquid chromatographic (HPLC) method with UV detector for the determination of dyclonine hydrochloride and a gas chromatography (GC) method with flame ionization detector (FID) for the determination of camphor and menthol in lotion were developed. The developed HPLC method involved using a SinoChoom ODS-BP C₁₈ reversed-phase column (5 μ m, 4.6 mm $\times$ 200 mm) and mobile phase consisting of acetonitrile : water : triethylamine in a ratio of 45 : 55 : 1.0; pH was adjusted to 3.5 with glacial acetic acid. The developed GC method for determination of camphor and menthol involved using an Agilent 19091J-413 capillary chromatographic column (30 m $\times$ 320 μ m $\times$ 0.25 μ m). The two methods were validated according to official compendia guidelines. The calibration of dyclonine hydrochloride for HPLC method was linear over the range of 20–200 μ g/mL. The retention time was found at 6.0 min for dyclonine hydrochloride. The calibration of camphor and menthol of GC method was linear over the range of 10–2000 μ g/mL. The retention time was found at 2.9 min for camphor and 3.05 min for menthol. The proposed HPLC and GC methods were proved to be suitable for the determination of dyclonine hydrochloride, camphor, and menthol in lotion.

1. Introduction

Dyclonine hydrochloride (Figure 1(a)), chemically 1-(4-butoxyphenyl)-3-1-piperidinyl-1-ropanon hydrochloride is belonging to a bactericidal and fungicidal local anesthetic. It has already become an important drug in the treatment of alleviation pain and antipruritic [1, 2]. Menthol (Figure 1(b)), chemically (1R,2S,5R)-2-isopropyl-5-methylcyclohexanol, is an organic compound made synthetically or isolated from *Mentha haplocalyx* Briq. which is an important medicine documented in Chinese Pharmacopoeia and has been widely used as an antipyretic and analgesic agent for treatment of headaches and itching [3, 4]. Camphor (Figure 1(c)), chemically 1,7,7-trimethyl bicyclo [2.2.1] heptan-2-one, is isolated from *Cinnamomum camphora* (L.) Sieb. which is mainly distributed in the south of the Yangtze River, and now it can be synthesized by chemist. It was used for agitation of the central nervous system and enhancement of the respiratory and circulatory system. It could also be used for alleviating pain and antipruritic [5–9].

The compound lotion is prepared with dyclonine hydrochloride, menthol, and camphor. The preparation is accepted by patients for many advantages such as less grease, skin cool, and so on. To the best of our knowledge, the current assay method was mainly focused on dyclonine hydrochloride; few methods have been developed in the literature for the estimation of dyclonine hydrochloride, menthol and camphor in preparations [10, 11]. The aim of this study is an attempt to develop and validate an HPLC method for dyclonine hydrochloride and a GC method for menthol and camphor with the advantages of shorter retention time and run time.

2. Experimental

Pure dyclonine hydrochloride, camphor, and menthol used as working standards were received as gifts from Zhengzhou Yonghe Pharmaceuticals Co., Ltd., Zhengzhou, China. Acetonitrile of HPLC grade and other chemicals of AR grade were purchased from Mingshen Chemicals Co., Ltd., Zhengzhou,

FIGURE 1: Typical chemical structures of (a) dyclonine hydrochloride, (b) menthol, and (c) camphor.

China. Deionized water was obtained by using a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.1. Preparation of Standard Stock Solution and Working Solution. The standard solution of dyclonine hydrochloride was prepared by dissolving accurately 10 mg pure drug in a 10 mL volumetric flask using mobile phase. The solution was sonicated for 5 min and then made up to the mark with mobile phase. Intermediate and working solutions were prepared by diluting stock solutions with the mobile phase.

The combined standard solution of menthol and camphor was prepared by dissolving accurately 10 mg menthol and 10 mg camphor in a 10 mL volumetric flask using chloroform. The solution was sonicated for 5 min and then made up to the mark with chloroform. Intermediate and working solutions were prepared by diluting stock solutions with chloroform.

2.2. HPLC Determination Conditions. Chromatographic analyses were performed using a Shimadzu system that was comprised of an LC-20AT pump, SPD 20A UV-Visible absorbance detector connected to Shimadzu Spin Chrome software. ODS-BP C₁₈ column (5 μ m, 4.6 mm $\times$ 200 mm) was used and the sample injection was performed via a Rheodyne syringe.

The mobile phase was a mixture of acetonitrile : water : triethylamine in a ratio of 45 : 55 : 1.0. The pH of mobile phase was adjusted to 3.5 with glacial acetic acid. The flow rate was 1.0 mL/min. The mobile phase was degassed by an ultrasonic bath and filtered through a 0.45 μ m membrane filter under vacuum. Wavelength was set at 280 nm. Flow rate was kept at 1.0 mL/min. The column was maintained at 35°C and injection volume was 20 μ L.

2.3. GC Determination Conditions. The GC method developed for determination of camphor and menthol was performed with Agilent 7890A and separated on Agilent 19091J-413 fused capillary chromatographic column (30 m $\times$ 320 μ m $\times$ 0.25 μ m) which was coupled to an FID detector. The temperature of detector and inlet was maintained at 250°C. The oven temperature was programmed at 250°C for 1 min, then 5°C/min to 170°C, and then hold at 170°C for 5 min. The split ratio was 10 : 1. The carrier gas was nitrogen

(99.99% purity) with a flow rate of 1.5 mL/min, and the analyzed sample volume was 1 μ L.

2.4. Method Validation. The method was validated as per ICH guidelines for specificity, linearity, accuracy, precision, and reproducibility.

2.4.1. Specificity. Dyclonine hydrochloride working solution (20.0 μ g/mL) and excipients sample working solution (including camphor 40.0 μ g/mL and menthol 40.0 μ g/mL) were scanned from 200 nm to 400 nm, and then the chromatograms were recorded with HPLC system.

Combined working solution (including camphor 80.0 μ g/mL and menthol 80.0 μ g/mL) and excipients sample working solution (including dyclonine hydrochloride 40.0 μ g/mL) were recorded with GC system.

2.4.2. Linearity. The linearity of the HPLC method for dyclonine hydrochloride was determined at seven concentration levels. The linearity of the GC method for camphor and menthol was determined at ten concentration levels. The responses were measured as peak areas.

2.4.3. Accuracy. A standard addition method was employed for accuracy experiment. Adequate amounts of dyclonine hydrochloride, camphor, and menthol corresponding to 80%, 100%, and 120% of the claimed concentration levels were added to excipients. At each level, three determinations were performed and the results were recorded. Accuracy was expressed as percent analyte recovered by the two proposed methods.

2.4.4. Precision. The precision of the two methods was checked by repeatability of injection, repeatability (intraday), intermediate precision (interday), and reproducibility. The results were expressed as the percentage relative standard deviation (% RSD) for ten determinations of peak areas of dyclonine hydrochloride (20.0 μ g/mL) and camphor and menthol (80.0 μ g/mL) performed. The same solutions were injected in triplicate for both intraday and interday variation.

2.5. Method Applications. 1 mL compound lotion was accurately transferred into another 50 mL volumetric flask and

FIGURE 2: Typical HPLC chromatograms of dyclonine hydrochloride ($20.0\text{ }\mu\text{g/mL}$), excipients sample working solution (including camphor $40.0\text{ }\mu\text{g/mL}$ and menthol $40.0\text{ }\mu\text{g/mL}$).

FIGURE 3: Typical GC chromatograms of camphor ($80.0\text{ }\mu\text{g/mL}$) and menthol ($80.0\text{ }\mu\text{g/mL}$), excipients sample working solution (including dyclonine hydrochloride $40.0\text{ }\mu\text{g/mL}$).

made up to volume with mobile phase to yield concentrations of $100\text{ }\mu\text{g/mL}$ for dyclonine hydrochloride. A $20\text{ }\mu\text{L}$ volume of the sample solution was injected into the chromatographic system three times under optimized HPLC conditions. The concentrations in the samples were determined by interpolation from calibration plots of dyclonine hydrochloride previously obtained.

1 mL compound lotion was accurately transferred into another 10 mL glass tube, and 5 mL chloroform was used to dilute the solution. The mixture was shaken for 2 min and centrifuged at 3000 rpm for 5 min. The above procedure was repeated twice. The supernatant was combined and evaporated to dryness at 50°C in bath water. The residue was dissolved with 10 mL chloroform. A $1\text{ }\mu\text{L}$ volume of the sample solution was injected into the chromatographic system three times under optimized GC conditions. The concentrations in the samples were determined by interpolation

from calibration plots of camphor and menthol previously obtained.

3. Results and Discussion

3.1. Specificity. The chromatographic conditions were optimized. According to the ultraviolet spectroscopy, dyclonine hydrochloride has maximum absorbance at 280 nm. Thus, 280 nm was selected as detected wavelength. Under the optimum conditions, typical chromatograms of dyclonine hydrochloride, camphor, menthol, and blank excipients are shown in Figures 2 and 3. The analyte peaks were well resolved and free from tailing (<1.5 for all analytes). The excipients in the compound lotion did not disturb the detection in the two methods. The retention time of dyclonine hydrochloride was found to be 6.0 min in HPLC chromatogram. The retention times of camphor and menthol

were found to be 2.9 min and 3.05 min in GC chromatogram.

3.2. Linearity. Under the optimal conditions, the HPLC method was linear over the range 20–200 $\mu\text{g/mL}$ for dyclonine hydrochloride. The regression equation $Y = 67.94X - 157.56$ was established based on the standard samples injected and their peak area with correlation coefficient of 0.9999.

The GC method was linear over the range 20–2000 $\mu\text{g/mL}$ for camphor and menthol. The regression equation $Y = 1.7359X - 39.406$ for menthol and $Y = 1.5687X - 35.544$ for camphor was established based on the standard samples injected and their peak area with correlation coefficient of 0.9991 and 0.9993.

The results indicate an excellent correlation between response factor and concentration of the three drugs.

3.3. Accuracy. The mean recovery data from the study for dyclonine hydrochloride was 99.92% in HPLC method, while they were 99.47% and 100.05% for camphor and menthol in GC method.

3.4. Precision. The precision study of intra- and interday variability was performed to determine the precision of the two developed methods. The RSD of intraday variation was 0.66% and the RSD of interday variation was 0.73% for dyclonine hydrochloride, respectively. The injection repeatability value of dyclonine hydrochloride was 0.89% in HPLC method.

The RSD of intraday variation was 0.49% and the RSD of interday variation was 0.51% for camphor; they were 0.72% and 0.83% for menthol, respectively. The injection repeatability values were 0.63% and 0.66% for camphor and menthol in GC method, respectively.

The results indicate that the two methods are precise and reproducible.

3.5. Method Applications. The validated two methods were applied to the determination of dyclonine hydrochloride, camphor, and menthol in compound lotion. The results of the assay yielded $99.43 \pm 0.28\%$, $100.08 \pm 0.36\%$, and $99.57 \pm 0.45\%$ for dyclonine hydrochloride, camphor, and menthol, respectively. It showed that the two methods were selective for determination of dyclonine hydrochloride, camphor, and menthol without interference from the excipients used in the compound lotion.

4. Conclusion

It is concluded from the above study that the two developed methods were simple, high sensitive and provide good reproducibility and accurateness for the determination of dyclonine hydrochloride, camphor, and menthol in compound lotion.

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