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ISOLATION OF HERNIARIN FROM MATRICARIA CHAMOMILLA EXTRACTIONS. APPLICATION TO DETERMINE IN CHAMOMILE AND LIPOSOMAL GEL

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Abstract

Chamomile (*Matricaria chamomilla L.*) is a medicinal plant species from the Asteraceae family. It is known to have numerous pharmacological activities, such as hypoglycemic, antistress, analgesic, anti-inflammatory, and antibacterial activities. This study was designed to isolate and quantitative herniarin, a well-known coumarin of chamomile. Herniarin was isolated from the chamomile absolute by vacuum liquid chromatography with n-hexane and chloroform mixed. Structure of herniarin was determined by analyzing the UV-Vis, IR, and NMR spectroscopy data. Herniarin quantitative procedures from chamomile extract and liposomal gel use HPLC system with DAD detector. This procedure was validated with the specificity, linearity, range, accuracy and precision characteristics.

Key words: *Matricaria chamomilla L.,* herniarin, liposomal gel, high performance liquid chromatography.
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1. Introduction

Chamomile *(Matricaria chamomilla L.)* is a medicinal herb in Europe. Main active constituents present in the plant are volatile oil (mainly chamazulene, farnesene, α-bisabolol), flavonoids (apigenin and its derivatives) and coumarins. Seven coumarins were described and herniarin is the highest amounts in plant extracts. This plant is used in pharmaceutical products and especially in cosmetics. Some of its therapeutical effects: anti-inflammatory, antiseptic, antimycotic, hemostatic, diuretic... It's also applied to wounds slow to heal, for skin eruptions, for hemorrhoids and for inflammation of the mouth, throat, and the eyes. Chamomile is used in perfumery, cosmetic creams, hair preparations, skin lotions, toothpastes, and also in liquors.

Liposomes are a form of vesicles that consist either of many, few or just one phospholipid bilayers. It helps to prevent harmful side-effects and degradation of drug, to increase the amount of the drug accumulated in the targeting zone. Recent researches have applied the liposomal technique to prepare the gel form from *Matricaria chamomilla* extracts using for dermatitis. Thus, the aim of our study will develop a high performance liquid chromatography method for determination of herniarin, was isolated from chamomile extracts, in the liposomal gel as a marker for *Matricaria chamomilla.*

2. Materials and Methods

2.1. Instrumention

Experiments were performed on a HPLC system of Agilent 1260 equipped with an auto sampler and Nucleosil C_{18} column (250 x 4.6 nm, 5 μm) coupled with temperature control system for column (40^0C) in the Laboratory of analytical chemistry and drug quality control department, pharmacy faculty, Lac Hong University.

500 MHz ^{1}HNMR and 125 MHz ^{13}CNMR were recorded in $CDCl_3$ on a Bruker instrument.

2.2. Chemicals and materials

Solvents for HPLC were acetonitrile (J.T.Baker) and deionised water. All chemicals and reagents used in this study were at analytical grade. Chamomile extract of flowers was acquired from Sigma - Aldrich. Herniarin were isolated from chamomile extract and its purity was assessed by HPLC-DAD and NMR as $\geq$99%.

2.3. Isolation of herniarin from chamomile extract

The chamomile extract was fractionated by vacuum liquid chromatography and eluted with solvent mixtures consists of n-hexane and chloroform. The following fractions were collected and tested by TLC. Fractions were evaporated out of solvents. Recrystallization technique was used to yield purity contents. A HPLC method with DAD detection assesses the purification of compounds: Nucleosil C18 column, 250 x 4.6 nm, 5 μm, mobile phase A (acetonitrile) and mobile phase B (deionised water), UV detection was at 254 nm, injection volume 20 μl, flow 1 ml/1 min.

The structure of compounds were determined by 1D (^{1}H, ^{13}C) and 2D (COSY, NOESY, HSQC, HMBC) NMR spectroscopy. The chemical shifts were expressed relative to an internal standard (TMS).

2.4. Quantification of herniarin in chamomile extract and liposomal gel

Sample preparation. Approximately 0.2g of chamomile extract was extracted with methanol at a concentration of 200 μg/ml under the ultrasonic wave for 10 min.

The pharmaceutical sample was prepared by dissolving in a 10 ml volumetric flask an accurately weighed amount of approximately 2 g of liposomal gel, added 6 ml of deionised water, under the ultrasonic wave for 15 min. Added 1 ml of Triton X-100 1/10 solution, then filled to the assigned volume by deionised water.

A solution of standard 3 μg/ml was prepared by dissolving herniarin in methanol.

All solutions were filtered through a 0.45 μm filter and a 20 μl of the solution were injected for analysis.

HPLC-DAD conditions. HPLC separation was performed on a reversed phase column Nucleosil C_{18}, 250 x 4.6 nm, 5 μm. Mobile phase A (acetonitrile) and mobile phase B (deionised water) were used in a gradient programme with a flow of 1 ml/min. The volume of single injection was 20 μl. After each injection, the column was re-equilibrated for 5 min. The wavelength of DAD detection was 320 nm. UV spectra between 260 and 400 nm were recorded to verify the peak's purity.

Method of validation. The HPLC method was subsequently validated according to the ICH guideline (ICH 2005) with respect to specificity, linearity range, accuracy, and precision.

Results and Discussions

3.1. Isolation of herniarin

The chamomile extract was fractionated by vacuum liquid chromatography. Twelve fractions were collected. Fractions were evaporated out of solvents. One of the fractions appeared a precipitate. Recrystallized with n-hexane, obtained a white crystalline compound. The chromatogram of this compound had only one peak when assessed the purification of the compound by a HPLC method. Combination UV, IR and NMR analyses allowed confirmation of herniarin.

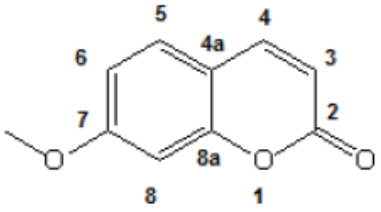

Figure 1. Structure of herniarin

UV spectrum: strong absorption bands at 210-220 nm and 320-325 nm. IR spectrum: herniarin showed strong bands at 1705 cm^{-1} for ketonic, 1612 cm^{-1} for C=C, 1506 and 1580 cm^{-1} for aromatic structure.

NMR spectrum: 1H NMR ($CDCl_3$, 500 MHz) δ 7.64 (1H, d, J = 9.5 Hz, H-4), 7.37 (1H, d, J = 8.5 Hz, H-5), 6.85 (1H, dd, J = 8.5, 2.0 Hz, H-6), 6.82 (1H, d, J = 2.5, H-8), 6.25 (1H, d, J = 9.5 Hz, H-3), 3.88 (3H, s, OMe); ^{13}C NMR ($CDCl_3$, 125.788 MHz) δ 162.87 (C, C-2), 161.18 (C, C-7), 155.96 (C, C-8a), 143.38 (CH, C-3), 128.74 (CH, C-5), 113.14 (CH, C-6), 112.6 (CH, C-8), 112.56 (C, C-4a), 100.89 (CH,C-4), 55.78 (CH_3, OMe).

3.2. Quantification of herniarin in chamomile extract and liposomal gel

The HPLC method was used to analyse herniarin in the chamomile extract. The gradient programme was used: 0-8 min 36% A; 8-22 min 48% A; 22-27 min 65% A; from 65 to 100% A in 2.0 min and finally kept at 100% A for 9.0 min. Figure 2 shows the HPLC-DAD chromatogram at 320 nm of the chamomile extract. Herniarin was detected at the retention time of 9.66 min. The retention time of standard compound is approximate with that of the respective peak obtained by analyzing samples.

System suitability was tested by performing six replicate injections. The RSD% values of the peak area and retention time for herniarin peak were

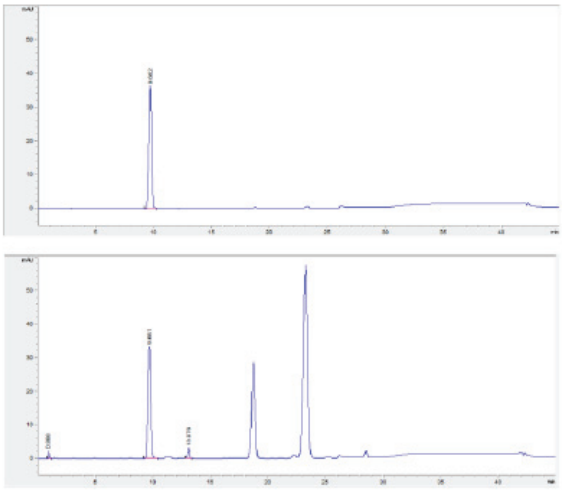

Figure 2. HPLC chromatogram of herniarin in standard and chamomile extract samples

less than 2%. Calibration curves were made based on the duplicated analysis of six concentration levels ranging from 1.0 to 6.0 μg/ml for herniarin with R^2 =0.9999. The recovery rates of herniarin were evaluated by spiking compound with final concentrations of 2.40, 3.00 and 3.60 μg/ml respectively with chamomile extract samples. The accuracy of the method expressed as recovery (%) was between 98 and 102%. Intra-day precision (repeatability) was evaluated by measuring six different samples at the same

concentration under the same experimental conditions on the same day. Inter-day precision (intermediate precision) was calculated from results obtained by the analysis of samples on two different days. The intra-day and inter-day relative standard deviation values obtained by the proposed method were found to be lower than 2.0%. Tests showed that there is no statistically significant difference.

The HPLC method was used to analyse herniarin in the liposomal gel. The gradient programme was used: 0-10 min 33% A; 10-25 min 48% A; 25-32 min 65% A; from 65 to 100% A in 2.0 min and finally kept at 100% A for 9.0 min. Figure 3 shows the HPLC-DAD chromatogram at 320 nm of liposomal gel. Herniarin was detected at the retention time of 11.26 min. The retention time of standard compound is approximate with that of the respective peak obtained by analyzing samples. There was no interference with the peak of herniarin in placebo sample.

The RSD% values of the peak area and retention time for herniarin peak were less than 2%. Calibration curves ranged from 0.55 to 1.65 g/ml with R^2 = 0.9998. The recovery rates of herniarin were evaluated by spiking compound

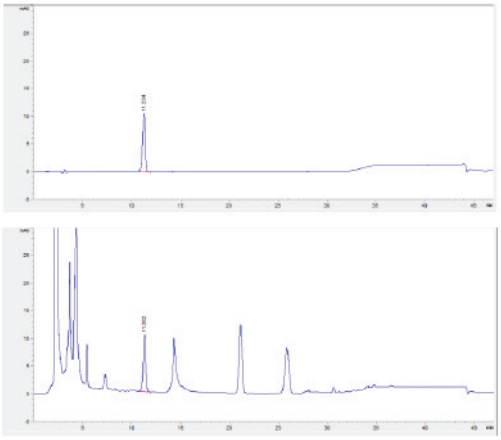

Figure 3. HPLC chromatogram of herniarin in standard and liposomal gel samples

with final concentrations of 0.88, 1.10 and 1.32 μg/ml respectively with placebo samples. The accuracy of the method expressed as recovery (%) was between 98 and 102%. The intra-day and inter-day relative standard deviation values obtained by the proposed method were found to be lower than 2.0%. Tests showed that there is no statistically significant difference.

Conclusions

In this study, herniarin was isolated from chamomile extract by the vacuum liquid chromatography. The structure of this compound was determined by UV, IR, NMR analyse data. Herniarin was pure and can be used as a standard for HPLC procedure. The study developed a simple HPLC method for quantification herniarin in the chamomile extract and in the liposomal gel with the wide range of the compound. All of the criteria were validated: specificity, linearity range, accuracy, and precision.

References

[1] A. Ahmad, L.N. Misra *Isolation of herniarin and other constituents from Matricaria chamomilla flowers* Inter. J. of Pharmacognosy, **35**(2) (1997), 121-125.

[2] Chao-Mei Ma, Linda Winsor, Mohsen Daneshtalab, *Quantification of spiroether isomers and herniarin of different parts of Matricaria matricarioides and flower of Chamaemelum nobile,* Phytochem. Anal. **18** (2007), 42-49.

[3] G. Haghi, A Hatami, A. Safaei and M. Mehran, *Analysis of phenolic compounds in Matricaria chamomilla and its extracts by UPLC-UV,* Res Pharma Sci. **9**(1)(2014), 31-37.

[4] ICH.Q2 (R1). "Validation of analytical procedures: Text and Methodology", 2005.

[5] Kant Shashi, Kumar Satinder, Prashar Bharat, *A complete review on liposomes,* Inter. research J. of Pharmacy, **7** (2012), 10-16.

[6] M.R. Sazegar et al., *The antioxidant activity of chamomile (Matricaria chamomilla L.) extracts in sunflower oil,* World applied sciences J., **9**(8)(2010), 873-878.

[7] Ompal Singh, Zakia Khanam, Neelam Mirsa, Manoj Kumar Srivastava, *Chamomile (Matricaria chamomilla L.): An overview,* Pharmacogn Rev. **5**(9) (2011), 82-95.

[8] Priyanka Kulkarni, Jaydeep Yadav, Kumar Vaidya, Liposomes, *A novel drug delivery system,* Inter. J. of current pharmaceutical Research, **3**(2011), 10-18.

[9] Veronika Preulova-Poracka, Microlav Repcak, Maria Vlikova, Jan Imrich, *Coumarins of Matricaria chamomilla L.: Aglycones and glycosides,* Food Chemistry **141** (2013), 54-59.