AN INVESTIGATION ON METHYLATION METHODS OF HESPERIDIN

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ABSTRACT

Since hesperidin is a poor water soluble compound, in pharmaceutical formulations its methylated derivatives (hesperidin methyl chalcone, HMC) are used. The aim of this study was to establish an efficient methylation method for preparation of hesperidin methyl derivatives. For this purpose hesperidin was isolated from tangerine peel, purified and its methyl derivatives were prepared using three different techniques, i.e. diazomethane, methyl iodide-sodium hydride and dimethylsulfate. The efficiency of the methods was evaluated in terms of the percentage of unchanged and intact hesperidin in the final methylated products the and amount of unchanged hesperidin was an indication of the better efficiency of the method. A reversed phase HPLC method was also developed for determination and quantification of hesperidin in the final methylated products .The method involved the use of a Shim pack CLC-ODS column, a mixture of methanol-phosphate buffer (37:63, v/v) of pH = 2.6 as a mobile phase in an isocratic mode at a flow rate of 1 ml/min and UV detection at 280 nm. The results showed that methylation with methyl iodide-sodium hydride have the highest efficiency among different methylation methods.

Keywords: Hesperidin, Hesperidin methyl chalcone, Citrus Reticulata, Tangerine

INTRODUCTION

Hesperidin, 3′, 5, 7-trihydroxy-4′-methoxy - flavanone-7- o - ß -rutinoside, is one of the bioflavonoids which is greatly found in Citrus species and is the major active constituent of tangerine peel (Citrus reticulata). Because of its poor aqueous solubility, the partially methylated derivatives of hesperidin have been synthesized. These derivatives which are called hesperidin methyl chalcone (HMC) are widely used in the preparation of pharmaceutical formulations which are used for modification of vascular permeability (1- 3), capillary resistance, microcirculation enhancer (4) and the relief of symptoms of acute hemorrhoid (5, 6). Meanwhile they possess anti-inflammatory (7), antihypertensive, diuretic (8), analgesic (9), hypocholestrolemic (10), anticancer (11) and antioxidant activities (12).

HMC is usually synthesized by methylation of hesperidin with dimethylsulfate in alkaline media. Reaction in alkaline media results in isomerization of hesperidin (flavanone), that is converted to hesperidin chalcone (chalcone) (13). Therefore a methylation reduction product consists of both methylated flavanones and chalcones which have been methylated in different degree. Thus, HMC is also a mixture of fully and partially methoxylated hesperidin which results from methylation of the hydroxyl substitutes on aglycon and linked sugars (figure 1) (14). .

Owing to the complex composition of HMC, the structures of the products of the reaction are not well known and many studies have been conducted on this subject. By HPLC of hesperidin methyl derivatives and analyses of their spectra by a diode array detector, eighteen derivatives have been reported (14). The authors demonstrated that HMC is a mixture of chalcone and flavanone species with an unspecified pattern of methylation and none of the derivatives were predominant product.

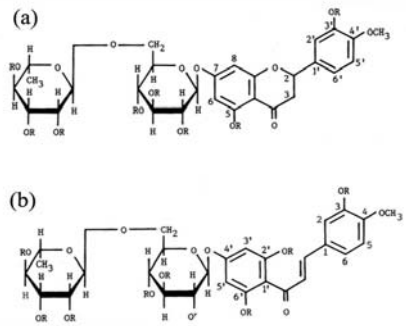

Figure 1. Structures of methylhesperidins. (a) Flavanone; (b) Chalcone. R = H or CH_3.

The aim of this study was to investigate the methylation yield of three available methods and to find out the best method having the highest yield for industrial applications. For this purpose,

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methods using methyliodide-sodium hydride, diazomethane and dimethylsulfate were carried out. The un-methylated hesperidin in the final product of the reaction was quantified by HPLC method and the less hesperidin in the final product was an indication of efficiency of methylation.

While there are several reports on the analysis of commercial hesperidin methyl chalcones using normal and reversed phase liquid chromatography with isocratic and gradient elution (14,15), no assay has previously been presented for the quantification of hesperidin in final products of methylation methods which was determined in this investigation.

MATERIALS AND METHODS

Chemicals:

All solvents and chemicals were of analytical grade except methanol that was HPLC grade and purchased from Merck chemical company (Darmstadt, Germany).

Hesperidin was isolated in our laboratory and its physical constants, spectral data and the results of HPLC analysis confirmed the purity of the product.

Apparatus

The HPLC system (Shimadzu, Japan) equipped with LC-8A pump, UV detector (Model SPD-6AV, Shimadzu), Shim pack CLC-ODS column (150 × 4.6 mm I.D., particle size of 5 µm, Shimadzu) and a rotary evaporator (Heidolph, Germany) were used.

Preparation of hesperidin

Hesperidin was extracted and purified from tangerine (Citrus reticulata) peels. An amount of 500 g air-dried and powdered tangerine peels was extracted three times with 1liter of water-methanol (30:70), by stirring and maceration for 8 hrs at ambient temperature. The extracts were filtered on the Whatman No. 1 filter paper and the solvent was removed by a rotary evaporator at 40 °C under vacuum. The residue was dissolved in water and the aqueous solution was successively extracted three times with 500 ml petroleum ether, chloroform, ethyl acetate and water-saturated n-butanol, respectively. Solvents of all fractions were removed by rotary evaporator under vaccumat 40 °C separately. The residues were dissolved in 50 ml of water-methanol (1:1) for TLC analysis. Hesperidin was found to be present mainly in the ethyl acetate extract of tangerine peel. Ethyl acetate solution was concentrated under vacuum at 40 °C to 150 ml and then was mixed with 50 ml of hexane. Hesperidin precipitated as a white brownish solid. The precipitates were filtered on the Whatman No.1 filter paper and washed with 50 ml acetone - water (1:1 v/v) in order to obtain pure hesperidin. At the end of the procedure, 4.2 g hesperidin was obtained as a white fine powder. Methylation of hesperidin was performed using three different methods according to the following procedures:

Methylation with dimethylsulfate (16)

To a solution of 500 mg hesperidin in 5 ml of 5% NaOH, was added 100 mg dimethylsulfate under continuous stirring during 8 hrs. The pH of final solution was adjusted to 5, kept overnight at this pH while stirring and then mixture was filtered. The aqueous solution was extracted three times by 30 ml of n-butanol. The extracts were evaporated by rotary evaporator to yield a yellow mass.

Methylation with methyl iodide-sodium hydride (17)

A mixture of 500 mg hesperidin in 30 ml dry dimethylformamide and 300 mg oil free sodium hydride was stirred under dry N_2 stream for 5 min. After stopping of the N_2 flow , 5 ml methyl iodide was added and the mixture was kept for 24 hrs in the dark place. The remaining sodium hydride was destroyed by careful addition of sufficient methanol. The resulting mixture was evaporated under reduced pressure and the residue dissolved in 20 ml of water. The aqueous solution was extracted twice with 30 ml of n-butanol to isolate methylated products and then the solvent was removed under vacuum to obtain a dry mass.

Methylation with diazomethane (16)

Diazomethane was prepared according to the reported method (16) and 10 ml of ethereal diazomethane solution was added dropwise to an ice-cooled solution of 200 mg hesperidin in 20ml of methanol. The mixture was kept in a tight glass at 0 °C for 5 min and then evaporated to dryness under vacuum.

TLC of methylated products

Products of methylation of each method was chromatographed on silica gel using ethyl acetate-methanol-water (100:17:13) and showed formation of several methylated derivatives. These products were more mobile than hesperidin and had different R_f values.

HPLC analysis

The following procedures were utilized for detection and quantification of hesperidin in different methylated products.

Preparation of standard solution

Five mg of purified hesperidin was dissolved in 15 ml of methanol and then was diluted to 50 ml by addition of methanol. The solution was filtered through 0.2 µm filter**.**

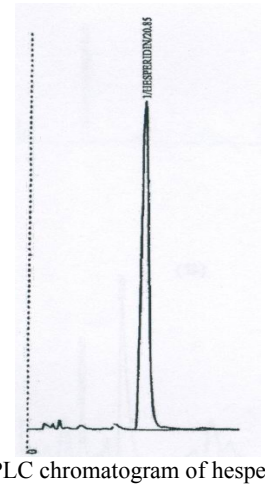

Figure 2. HPLC chromatogram of hesperidin standard.

Preparation of methylated derivatives

Fifty mg of each methylated product was dissolved in 50 ml of methanol and the pH of solution adjusted to 7 and the solution was filtered through a 0.2 µm filter.

HPLC analysis conditions

HPLC analysis was performed using methanol - phosphate buffer (10 mM) of pH =2.6 (37:63 v/v) as mobile phase and a 150×4.6 mm I.D. Shim pack CLC-ODS column with an average particle size of 5 µm. Analysis was run under isocratic elution with a flow rate of 1ml/min, at 25 °C and injection volume for all samples were 25 µl. The effluent was monitored at 280 nm.

Figure 2 shows the HPLC chromatogram of hesperidin. Figure 3 shows the HPLC chromatogram of remaining hesperidin in methylated products using, A) methyl iodide-sodium hydride, B) dimethylsulfate and C) diazomethane.

RESULTS AND DISCUSSION

Since hesperidin from natural sources produces very poorly water-soluble crystals, various methylation techniques were used to increase its solubility through changing its physical properties and preventing the ease of its crystallization. According to the literature, the most common method of methylation is the use of the dimethylsulfate (14). In addition to this method, diazomethane and methyliodide – sodium hydride methylation methods were performed in this investigation. As a quick qualitative survey of the performance of different methylation methods a TLC analysis was used. The results showed several methylated derivatives with different R_f values higher than hesperidinwhich were formed by different degree of methylation of the hydroxyl groups of hesperidin according to their acidities (16). While all three methylation methods increased the solubility of hesperidin, the most important point was to decrease the amount of un-methylated hesperidin as much as possible.

Table 1. Efficiency of different methods for methylation of hesperidin.

Method of methylation	Efficiency of methylation (%)
Diazomethane	93.9
Dimethylsulfate	96.3
Methyliodide-sodium hydride	98.8

* The amount of methylated derivatives has been considered as the efficiency of the method.

In order to quantify un-methylated hesperidin, a simple isocratic HPLC method was employed throughout this study. The results of HPLC analysis of methylated products revealed that a part of hesperidin remains as un-methylated in the reaction media. The amount of hesperidin in methylated products using diazomethane, dimethylsulfate and methyliodide–sodium hydride was 6.1, 3.7 and 1.2 % respectively (figure 3).

Our results showed that the methyliodide – sodium hydride provides the highest methylation performance and this method is recommended for industrial applications (Table 1). In addition to this finding, a HPLC method was developed for quantitative analysis of hesperidin in methylated products.

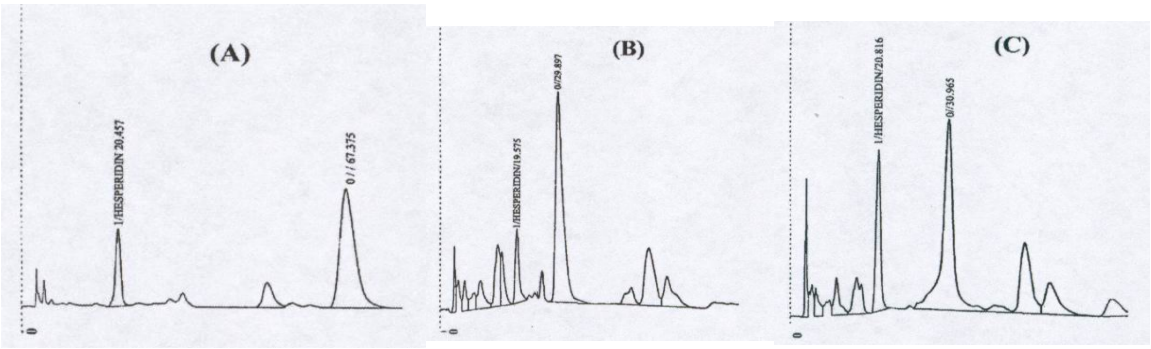

Figure 3. HPLC chromatogram of hesperidin analysis in three methylation products using: A, methyl iodide – sodium hydride; B, dimethylsulfate; C, diazomethane.

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