Bulletin of Faculty of Pharmacy, Cairo University xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Bulletin of Faculty of Pharmacy, Cairo University

journal homepage: www.elsevier.com/locate/bfopcu

Original Article

Validated determination of diacerein and its active metabolite, rhein, by stability indicating constant pattern method as a novel manipulation of zero order spectra

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ARTICLE INFO

Keywords:
Constant absorbance pattern
Diacerein
Rhein
Validation

ABSTRACT

A novel spectrophotometric method named constant absorbance pattern technique was proposed for simple determination of binary mixtures exemplified by diacerein, a treatment of osteoarthritis, and rhein, diacerein main alkaline degradation product and active metabolite. The proposed novel constant absorbance pattern technique was highly accurate, precise, sensitive, specific and stability indicating as well. The proposed technique allowed determination of diacerein and rhein in their binary mixtures manipulating zero order absorption spectra with no need for ratio spectra, derivatization steps, or even preliminary separation. Diacerein was determined at zero absorption spectra depending on the constant absorbance pattern between the two wavelengths 230 and 430 nm while rhein was directly determined at zero order at its λ_{max}, 430 nm. The proposed methods showed obeying for Beer-Lambert's law in the concentration range of 1–16 μg mL^{-1} for both diacerein and rhein. Validation of the proposed methods was carried out according to ICH guidelines with respect to accuracy and precision. Moreover, the proposed method was applied for determination of Diacerein in different pharmaceutical formulations and results were statistically compared with those of the reported HPLC one where no significant difference was found concerning Student's *t*-test and F-value.

1. Introduction

Diacerein (DIA), 1,8-diacetoxy-3-carboxyanthraquinone, [1,2] Fig. 1a, is a treatment for osteoarthritis and vascular diseases [3]. Rhein (RH), 4,5-dihydroxyanthraquinone-2-carboxylic acid [2], Fig. 1b, is the active metabolite [2] and the hydrolytic degradation product of DIA [4]. RH activates the synthesis of proteoglycans and hyaluronic acid, cartilage components, and inhibits synthesis of interleukin-1, cytokine involved in cartilage damage [5]. It can be easily synthesized from aloin, the natural glucopyranoside [4].

No official methods were reported in the literature survey for DIA determination either in pharmaceutical formulations or in its bulk powder. In contrast, some methods for its determination either alone or in combination with other drugs were reported such as, spectrophotometric [4,6–15], flow injection chemiluminescence [16], colorimetric [17–21], and RP-HPLC [22–25] methods. On the other hand, DIA has been selectively determined in presence of its degradation product by HPLC [26–29] and TLC-Densitometric [4,30] methods with no quantification of RH. Another TLC-Densitometric method was reported for its determination along with its degradation product and major impurity, emodin [31].

As per ICH guidelines Q_1 A (R_2) [32] on stability testing of new drug substances and products, DIA was subjected to stress conditions as hydrolysis, oxidation, and photolytic degradation. Hydrolysis and oxidation were degrading conditions for DIA where RH was the main product, while DIA was stable under photolytic degradation conditions [31]. RH was reported to be the active metabolite of DIA as well [2].

Spectrophotometric methods are preferable in some laboratories lacking of expensive chromatographic facilities as in developing countries. So, it is a big challenge for analysts resolving complex mixtures with accurate, precise, and reproducible results via applying simple spectrophotometric methods. Spectrophotometric methods manipulating zero order absorption spectra are the most preferable ones that there is no need for derivatization or complex data manipulation.

Diacerein and RH showed partial spectral overlap, due to similarity in their chemical structure, which hindered determination of both DIA and RH by simple spectrophotometric methods. The reported spectrophotometric methods [6–15], allowed determination of DIA only either alone or in combination with other drugs. Only one method [4] was reported for determination of DIA in presence of RH without RH

Peer review under responsibility of Faculty of Pharmacy, Cairo University.
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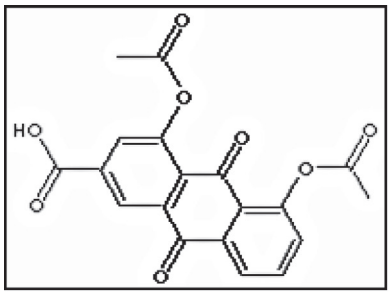
https://doi.org/10.1016/j.bfopcu.2017.11.005
Received 7 August 2017; Received in revised form 24 October 2017; Accepted 22 November 2017

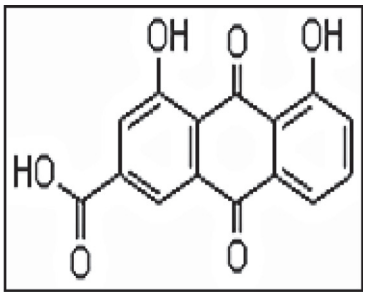
Please cite this article as: Emam, A.A., Bulletin of Faculty of Pharmacy, Cairo University (2017), https://doi.org/10.1016/j.bfopcu.2017.11.005



Molecular Formula: $C_{19}H_{12}O_8$

M. Wt. 368.29

(a)



Molecular Formula: $C_{15}H_8O_6$

M. Wt. 284.22

(b)

Fig. 1. Chemical structure of Diacerein (a) and Rhein (b).

quantification using the complex derivative and derivative ratio spectrophotometric methods.

As a result; and due to DIA instability and being RH the active metabolite and main degradation product [2,4,31], it was essential to determine both DIA and RH with simple spectrophotometric methods manipulating zero order spectra. The spectral overlap between their absorption spectra was the challenge part during their determination due to their chemical structural similarity.

So, the aim of the presented work was to develop a stability indicating simple spectrophotometric methods manipulating zero order spectra without derivatization or preliminary separation. The proposed constant absorbance pattern technique can be used as dependable and alternative ones for the reported derivative and derivate ratio spectrophotometric methods [4] for quality control of DIA in bulk powder and pharmaceutical formulations.

1.1. Theory of constant pattern method (CP)

Depending on the fact that; for a spectrum of a certain component (X); a constant pattern is followed by different concentrations so, the ratio of the response (absorbance) between two certain wavelengths (λ_1 and λ_2) is constant for different concentrations [33] so, a mixture of two components, X and Y, with overlapped spectra can be determined manipulating their zero order spectra.

$$A_{X1}/A_{X2} = \text{Constant} \quad (1)$$

where A_{X1} and A_{X2} are the absorbance values of component X at two wavelengths (λ_1 and λ_2), respectively.

Knowing that component X is more extended than component Y at λ_2 so,

$$A_{mix1} = A_{X1} + A_{Y1} \quad (2)$$

$$A_{mix2} = A_{X2} \quad (3)$$

where, A_{mix1} and A_{mix2} are absorbance values of the mixture at λ_1 and λ_2, respectively, A_{Y1} is the absorbance value of component Y at λ_1.

Dividing (2) by (3);

$$A_{mix1}/A_{mix2} = (A_{X1} + A_{Y1})/A_{X2} \quad (4)$$

$$A_{mix1}/A_{mix2} = A_{X1}/A_{X2} + A_{Y1}/A_{X2}$$

From (1), A_{X1}/A_{X2} = Constant so,

$$A_{mix1}/A_{mix2} = \text{Constant} + A_{Y1}/A_{X2}$$

As A_{mix1}/A_{mix2}, Constant, and A_{X2} can be obtained from the spectrum of a mixture; so, A_{Y1} can be calculated as follows

$$A_{Y1} = [(A_{mix1}/A_{mix2}) - \text{Constant}] \times A_{X2} \quad (5)$$

So, component Y concentration in the mixture can be calculated using a corresponding calculated regression equation for pure Y component at (λ_1). Similarly, component X concentration can be directly calculated from any λ_{max} (λ_2) at the extended region without any interference from Y using its corresponding calculated regression equation.

2. Experimental

2.1. Instruments

A double beam UV-1601 PC UV–visible spectrophotometer (SHIMADZU, Japan) with 1 cm matched quartz cell connected to IBM compatible computer. Spectra were automatically obtained by UVPC spectroscopy V. 3.7 software. The spectral bandwidth was 2 nm and wavelength-scanning speed 2800 nm min^{-1}.

2.2. Materials and reagents

2.2.1. Pure standards

A pure standard of diacerein was supplied as a gift by Delta Pharm Co. (Kafr El Gabal – Haram – Giza, Cairo, Egypt) with purity of 99.69 as per manufacturer certificate.

2.2.2. Pharmaceutical formulations

1-Diacerein® capsules: manufactured by Delta pharm Co. B. No. 004497, labeled to contain 50 mg of DIA per capsule.

2-Osteocerein® capsules: manufactured by Novartis pharma Co. (S.A.E Cairo). B. No. Y 0017, labeled to contain 50 mg DIA per capsule.

2.2.3. Chemicals and solvents

All chemicals and solvents used throughout this work were of analytical grade and were used without further purification; ethyl acetate, hexane, HCl, and NaOH (El-Nasr for Pharmaceutical Chemicals Co., Abu Zabaal, Cairo, Egypt); acetonitrile, acetic acid, and methanol HPLC grade (CHROMOSOLVE® Sigma-Aldrich Chemie GmbH, Germany).

2.2.4. Degraded sample

100 mg of pure DIA were accurately weighed and transferred into a 100 mL flask containing 50 mL 0.1 N NaOH solution and refluxed for 5 h till complete degradation was confirmed via TLC-densitometry using (60:40:0.8, by volume) of hexane, ethyl acetate, acetic acid as a developing system where only one spot was observed not corresponding to DIA spot [31]. 0.1 N HCl solution was gradually added till neutralization and complete precipitation where the precipitate was