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A comparative study of smart spectrophotometric methods for simultaneous determination of a skeletal muscle relaxant and an analgesic in combined dosage form

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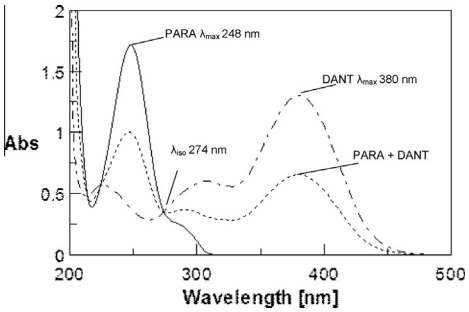
HIGHLIGHTS

- Six different spectrophotometric methods based on isosbestic point or ratio spectra.
- All methods could be applied for simultaneous analysis of PARA and DANT.
- All methods could be easily applied in QC labs.
- No significant difference in accuracy and precision compared to reported method.
- They are applied for analysis of the dosage form without interference of excipients.

GRAPHICAL ABSTRACT



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ABSTRACT

Six simple, specific, accurate and precise spectrophotometric methods were developed and validated for the simultaneous determination of the analgesic drug; paracetamol (PARA) and the skeletal muscle relaxant; dantrolene sodium (DANT). Three methods are manipulating ratio spectra namely; ratio difference (RD), ratio subtraction (RS) and mean centering (MC). The other three methods are utilizing the isoabsorptive point either at zero order namely; absorbance ratio (AR) and absorbance subtraction (AS) or at ratio spectrum namely; amplitude modulation (AM). The proposed spectrophotometric procedures do not require any preliminary separation step. The accuracy, precision and linearity ranges of the proposed methods were determined. The selectivity of the developed methods was investigated by analyzing laboratory prepared mixtures of the drugs and their combined dosage form. Standard deviation values are less than 1.5 in the assay of raw materials and capsules. The obtained results were statistically compared with each other and with those of reported spectrophotometric ones. The comparison showed that there is no significant difference between the proposed methods and the reported methods regarding both accuracy and precision.

Introduction

Paracetamol (PARA); N-acetyl-p-aminophenol; (Fig. 1a) is a widely used analgesic and antipyretic agent for the relief of fever, headaches and minor pains. It is a major ingredient in numerous cold and flu remedies. In combination with non-steroidal anti-

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inflammatory drugs and opioid analgesics; paracetamol is also used in the management of severe pain (such as post-operative pain) [1,2]. Paracetamol alone or in combination with other drugs was estimated by titrimetry [3,4], spectrophotometric method [5–7], HPLC [8,9], TLC [10], HPTLC [11], UHPLC [12], LC–MS [13], FT-IR [14], amperometric determination [15] and fluorimetry [16].

Dantrolene sodium (DANT); 1-[5-(4-nitrophenyl)furfurylidene amino]imidazolidine-2,4-dione sodium salt (Fig. 1b) is a muscle relaxant with a direct action on skeletal muscles [1]. DANT uncouples muscular contraction from excitation, probably by interfering with the release of calcium from the sarcoplasmic reticulum [1]. DANT was also determined either alone, in presence of its metabolites and impurities or in biological fluids utilizing different methods as HPLC [17–20] and polarography [21].

Most of the drugs used for treatment of skeletal muscle disorders are combined with analgesics such as PARA. PARA and DANT are formulated as capsule where this combination is used for myalgia, sprains and skeletal muscle spasms due to neurological disorder. Literature search revealed only two methods for the simultaneous determination of PARA and DANT in dosage form; HPLC-UV [22] and spectrophotometry based on zero order, first derivative and derivative ratio methods [23].

The main problem of spectrophotometric binary or ternary mixture analysis is the simultaneous determination of the compounds in the same mixture without prior separation. Several spectrophotometric methods have been used for resolving such mixtures with overlapped spectra such as; solving two simultaneous equations (Vierodet's method) [24], Derivative spectrophotometry [25], ratio derivative spectrophotometry [26,27], in addition to, H-point standard addition method [28].

Thus, the aim of this work was to develop and conduct comparative study on recently developed well established spectrophotometric methods based either on ratio spectra (RD, RS, MC) or on isosbestic point (AR, AS and AM) for resolving binary mixture of spectral interfering problem without preliminary separation. The utilized methods are very simple, accurate, precise and do not require any sophisticated apparatus.

Theory

Absorbance ratio method (AR)

Erk [29] developed a spectrophotometric method which is based on the linear relationship between the absorbance ratio value of a binary mixture of *X* and *Y* and the relative concentration of such a mixture. The main criteria for the application of this method are the existence of an isoabsorptive point and the selection of optimum wavelengths (λ_1, λ_2, λ_{iso}) which can fulfill good determination of the components in the binary mixture.

Absorbance subtraction method (AS)

This method is based on the same principles as the absorbance factor method [30–33]. However, this method could be applied for the analysis of a mixture of two drugs *X* and *Y* having overlapped spectra intersecting at isoabsorptive point where *Y* is extended over *X*, and *X* doesn't show any contribution at another wavelength (λ_2). In this method, the isoabsorptive point λ_{iso} could be used for separate quantitative estimation of each *X* and *Y* in their mixture (*X* + *Y*). The determination can be done using mathematically calculated factor of one of these components. By simple manipulation step, we can obtain the absorbance value corresponding to *X* and *Y*, separately. So, the concentration of each component could be obtained via the isoabsorptive point regression equation without any need for a complementary method.

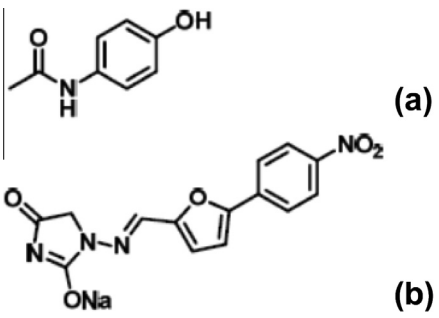

Fig. 1. Chemical structures of (a) paracetamol (PARA), (b) dantrolene sodium (DANT).

Amplitude modulation method (AM)

The method is a ratio spectrum manipulating method using the normalized spectrum of the divisor obtained by dividing certain spectrum of *Y'* component by its concentration [30,32]. For a mixture of *X* and *Y*, where *Y* is more extended than *X*; *X* and *Y* shows isoabsorptive point at the zero order spectrum and consequently is retained as an isosbestic point at the ratio spectrum.

Experimental

Apparatus and software

A JASCO V-530 double beam UV–VIS spectrophotometer loaded with Spectra Manager Program (JASCO) was used for spectral acquisition and elaboration of the data obtained. Quartz cuvettes, 1-cm pathlength were used for measuring the light absorption in the ultraviolet region (200–400 nm). For the mean centering for ratio spectra method, Matlab® Version 7.9 was used.

Chemicals

Pure samples: paracetamol (PARA) was kindly supplied by International Drug Agency for Pharmaceutical Industry (Portsaid, Egypt). Dantrolene sodium (DANT) was kindly supplied by Chemipharm Pharmaceutical Industries (6th October City, Egypt). Their purity was found to be 99.82 ± 0.71 and 99.83 ± 0.76 for PARA and DANT, respectively, by a reported method [23].

Market sample: Dantrelax compound® capsules labeled to contain 300 mg PARA and 25 mg DANT (Batch number: 131186A), was manufactured by Chemipharm Pharmaceutical Industries (6th October City, Egypt).

Solvents: methanol was obtained from Merck (Darmstadt, Germany).

Standard solutions

Separate stock solutions of PARA and DANT (1 mg/mL) were prepared by dissolving the compounds in methanol then completing in 100 mL measuring flasks. The working solutions were freshly prepared by dilution from the stock solutions with the same solvent to obtain a concentration of (0.1 mg/mL) for each of PARA and DANT.

Procedures

Spectral characteristics

The zero-order absorption spectra (D_0) of the two components were recorded at (200–400 nm) against methanol as a blank.

Construction of calibration curves

Ratio difference method (RD). Standard solutions containing 1–30 μg/mL PARA and 1–40 μg/mL DANT were prepared separately in methanol. The absorption spectra of the resulting