Bulletin of Faculty of Pharmacy, Cairo University (2016) xxx, xxx–xxx

Cairo University

Bulletin of Faculty of Pharmacy, Cairo University

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ORIGINAL ARTICLE

Simple spectrophotometric methods for the simultaneous determination of antipyrine and benzocaine

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Received 6 December 2015; revised 9 April 2016; accepted 14 May 2016

KEYWORDS

Antipyrine;
Phenazone;
Bezocaine;
Dual wave length;
Isoabsorptive point;
Different spectrophotometric method

Abstract Antipyrine and benzocaine are formulated together for the treatment of ear inflammation and to relieve pain. Four spectrophotometric methods were developed for the simultaneous determination of antipyrine (AN) and benzocaine (BE) in their combined dosage form. Method A depends on applying dual wavelength method where antipyrine was determined by measuring the absorbance at 254.1 and 309.1 nm (corresponding to zero difference of benzocaine), while the absorbance difference at 230.1 and 263.5 nm (corresponding to zero difference of antipyrine) was selected for benzocaine determination in the laboratory prepared spectrum. Method B depends on measuring the peak amplitude of first derivative at 305 nm for calculating benzocaine concentration then the total concentration of both drugs was determined using isoabsorptive point at 257.4 nm (antipyrine concentration was then calculated by subtraction). Method C is based on measuring the peak difference of the ratio spectra at Δp (239.1–285 nm) and Δp (301.4–250 nm) for the determination of antipyrine and benzocaine, respectively. Method D depends on measuring peak to peak amplitude of the first derivative of ratio spectra at (234.5 + 244.2 nm) and peak amplitude at 295.5 nm for the determination of antipyrine and benzocaine, respectively. The proposed methods were validated and applied for the analysis of antipyrine and benzocaine in their laboratory prepared mixtures and pharmaceutical formulation. Statistical comparison between the results of the proposed methods and those of the reported methods showed no significant difference.

1. Introduction

Antipyrine (phenazone) (AN, Fig. 1) is 1, 5-Dimethyl-2-phenyl-4-pyrazolin-3-one, it is a NSAID that can relieve mild to moderate pain.[1] It is an official drug in USP[2] and BP[3] pharmacopeia. Several analytical methods were reported for the determination of AN either alone or coformulated with other drugs including spectrophotometric,[4,5] chemometric,[6,7] HPLC,[8,9]

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Peer review under responsibility of Faculty of Pharmacy, Cairo University.

http://dx.doi.org/10.1016/j.bfopcu.2016.05.003

TLC,[10,11] GC,[12,13], capillary zone electrophoresis[14] and non aqueous titration[15] methods. Benzocaine (BE, Fig. 1) is a benzoic acid, 4-amino, ethyl ester (ethyl 4-aminobenzoate) acid ester. It is used as a local anesthetics for superficial anesthesia, for the local and temporal relief of pain related, to buccal infections.[1] BE is official in USP[2] and BP[3] pharmacopeia. BE was determined by several methods including; spectrophotometry[16–21], and HPLC.[22–26] HPLC method was also reported as a stability indicating method for BE.[27] Antipyrine (AN) and benzocaine (BE) are co-formulated in Egyptian market as ear drops for relief of pain and reduction of inflammation in the ear as in otitis externa and acute otitis media[15]. Usually combined dosage forms require more sophisticated analytical methods especially when their components interfere with each other during their analysis and control procedures to resolve the interference.[28] Only two HPLC methods were elaborated for the determination of benzocaine and antipyrine in the presence of BE degradation product.[29,30] One TLC method was reported for the determination of benzocaine and antipyrine in the presence of BE degradation product.[30] From literature in hand, no spectrophotometric methods were used for the simultaneous determination of AN and BE, therefore the aim of this work was to develop and validate simple, precise, rapid and accurate spectrophotometric methods for the simultaneous determination of AN and BE in pure form or in pharmaceutical formulation.

2. Experimental

2.1. Instruments

SHIMADZU dual beam UV–visible spectrophotometer (Kyoto/Japan), model UV-1650 PC with a fixed slit width (1 nm). The bundle software is UV-Probe version 2.21 (Shimadzu). The instrument is equipped with HP1020 laser jet printer.

2.2. Chemicals and solvents

All chemicals used were of analytical grade: Double distilled water (SEDICO Pharmaceutical Co., Cairo, Egypt). Ethanol (Lab. Scan, Ireland).

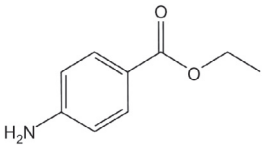
2.3. Samples

2.3.1. Pure samples

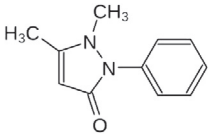
- Antipyrine (phenazone) and Benzocaine were kindly supplied by Amriya for pharmaceutical industries, Alexandria, Egypt. Purity of AN and BE was found to be 100.22 ± 1.375 and 99.47 ± 1.071 according to its reported method.[30]

2.3.2. Pharmaceutical formulation

- Otosept® ear drops are labeled to contain 300 mg of antipyrine and 100 mg of benzocaine in 10 mL of glycerin, It is manufactured by Amriya for pharmaceutical industries, (Alexandria, Egypt). Batch No. 857103[1] was purchased from Egyptian markets.



Benzocaine, $C_9H_{11}NO_2$, M.Wt 165.19



Antipyrine, $C_{11}H_{11}N_2O$, M.Wt 188.23

Figure 1 The chemical structures of benzocaine and antipyrine.

2.4. Standard solutions

2.4.1. Stock standard solutions

- Antipyrine (1 mg mL^{-1})
  The solution was prepared by dissolving 100 mg of AN in 100 mL double distilled water.
- Benzocaine (0.4 mg mL^{-1})
  The solution was prepared by dissolving 40 mg of BE in the least amount of ethanol then complete to 100 mL using double distilled water.

2.4.2. Working standard solutions of AN (100 μg mL^{-1}) and BE (40 μg mL^{-1})

AN and BE working solutions were prepared by diluting 10 mL from their respective stock standard solutions 1 mg mL^{-1} and 0.4 mg mL^{-1} (for AN and BE, respectively) into two separate 100-mL volumetric flasks and the volume was completed with double distilled water.

2.5. Laboratory prepared mixtures

Different aliquots of AN and BE were separately transferred from their working standard solutions (100 μg mL^{-1} and 40 μg mL^{-1}, respectively) into a set of 10-mL volumetric flasks then the volume was completed to the mark with double distilled water to produce laboratory prepared mixtures containing different ratios of the cited drugs.

3. Procedure

ICH guidelines[31] for method validation were followed.

3.1. Spectral characteristics

Zero order absorption spectra of 30 μg mL^{-1} AN and 10 μg mL^{-1} BE in double distilled water were recorded over a wavelength range of 200–400 nm.

3.2. Linearity

Aliquots of AN and BE were separately transferred from their working standard solutions (100 μg mL^{-1} and 40 μg mL^{-1}, respectively) into two separate sets of 10-mL volumetric flasks. The volume was then made up to the mark with double distilled water to give samples having concentrations in the range of 5–50 μg mL^{-1} for AN and 1–20 μg mL^{-1} for BE. The zero

Please cite this article in press as: Merey HA Simple spectrophotometric methods for the simultaneous determination of antipyrine and benzocaine, *Bulletin Facult Pharmacy Cairo Univ* (2016), http://dx.doi.org/10.1016/j.bfopcu.2016.05.003