Lbn Al Haitham Journal for Pure and Applied Science

Journal homepage: http// jih.uobaghdad.edu.iq/index.php/j/index

Colorimetric Determination of Meprobamate after a simple derivatization

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Article history: Received 9 January 2019, Accepted 17 February 2019, Publish September 2019.

Doi:10.30526/32.3.2283

Abstract

A sensitive and environmentally benign spectrometric method was developed for quantifying Meprobamate (MEP). The analyzed MEP was derivatized into a colored complex and determined spectrometrically. The colorimetric analytical parameters were optimized and validated. Low limit of detection (LOD) was achieved down to 1.88×10^{-6} mol/l while the limit of quantification (LOQ) was extended over the range of 1.97×10^{-6} - 1.35×10^{-3} mol/l. The high precision has been denoted by the 1.54% value of the coefficient of variation. The recovery was 96.07%, while the RSD (n=3) was 1.05 - 1.19%. The apparent molar absorptivity (Ɛ) obtained within 1154.7 - 1691.9 $L.mol^{-1}.cm^{-1}$. The outcomes have signified the high sensitivity of the technique. Furthermore, the avoidance of any traditional sample preparation steps prior to the analysis makes the method an excellent environmental approach. There were no analytical interferences with the common composites. The method has been successfully verified for the quantification of MEP content in bulk pharmaceutical matrix and can be applied to determine other environmentally toxic carbamates.

Keywords: Meprobamate, colorimetric, derivatization, Job's method, carbamate ester, Miltown.

1. Introduction

Paradin tablets, synthesized by the State Company for Drugs Industry & Medical Appliances/ Samarra Drugs Iraq (SDI), are composed of paracetamol, caffeine, and meprobamate (MEP) [1]. Meprobamate (also called Urethane, Miltown, "2-Methyl-2-propyl-1,3-propanediol dicarbamate"[2].), is a white powder of carbamate esters family [3]. with a p*Ka* of 9.2 [4]. soluble in acetone and alcohol, but it is insoluble in ethers [5]. Meprobamate solubility in water is of around ~ 4700 mg/ L [6]. It was used as an analgesic, muscle relaxant, anxiolytic, sedative, anticonvulsant action [7]. and to treat youngsters with cerebral paralysi [8]. As a muscle relaxant [9]. MEP has a varying narcotic consequence for which depends on its maltreatment potential predominantly [10]. Several reports have stated the poisoning and serious consequences of MEP, which may cause death [11]. Yet, it found to cause some

undesirable disorders, such as sedation, cerebral pains, dumbfounding energy, perplexity, psychological and psychomotor disability and disarray in elderly individuals [12]. Additionally, carbamate pesticides are highly toxic compounds. It can be detected in wastewater and may reach freshwater resources [13]. Self-poisoning of MEP has been described as conceivably coaxing hypotension[14]. Therefore, a reliable and easy quantitation method of MEP could be important in toxicology [15]. clinical and forensic labs [16]. Methods used for quantifying MEP such as gas chromatography-flame ionization detector (GC-FID) [17]. Mass spectroscopy (MS) [18]. or reverse phase-high performance liquid chromatography (RP-HPLC) are not sensitive enough. They showed low sensitivity for analyzing carbamates due to weak response of the detector, thus, it should be derivatized first before analyzed by GC-FID or MS. Meprobamate was identified spectrometrically by forming a complex with a tetra-substituted benzoquinone[19]. favorably with the tetrahalo derivative which is known as *p*-chloranil or bromanil[20]. The subsequent colored charge transfer (CT) complex[21]. can be detected colorimetrically [22]. Mass spectroscopy is commonly used to quantify MEP after concentrating the sample using implementing solid phase extraction SPE technique [23]. Using RF-HPLC method to separate and identify MEP [24]. was not recommended without using appropriate derivatization since meprobamate showed no convenient UV absorption [25].

The simplicity of colorimetry makes it the best approach to quantify MEP, where it reacts selectively with a colored reagent to produce a colored derivative can be easily measured [26].

2. Experimental

2.1. Instruments

A double beam spectrophotometer UV/Visible, T80+ (PG Instruments) was used to measure the absorbance. A differential scanning calorimeter (DSC) model STA PT1000 (Linseis, Germany) was utilized for the thermal analyses.

2.2. Methods

Acid mixture: was prepared by adding 0.5mL of concentrated hydrochloric acid (~12.08M) to 9.5 mL of concentrated sulfuric acid (~17.82M) with continuous stirring. A stock solution of MEP: A 0.005 g of MEP was dissolved in 10 mL of a freshly prepared acidic mixture (0.5 mL HCL and 9.5 mL H_2SO_4) with a final concentration of (2.3×10^{-3} mol/L).

Working solutions of MEP: The stock solution was diluted using the acidic mixture to prepare sets of standard working solutions.

Preparation of p-Dimethylaminobenzaldehyde (p-DMA) solution: A 0.0034 g of P-DMA was dissolved in 10 mL of the freshly prepared acidic mixture to obtain (2.3×10^{-3} mol/L) solution.

The calibration curves: A 0.5 mL of the freshly prepared *p*-DMA was added to a series of concentrations of MEP solution (1.97×10^{-6} – 1.35×10^{-3} mol/L) and left at 120 ℃ for 25 min. Each standard solution was then transferred into an ice bath, followed by the addition to 1 mL of cold distilled water. The absorptions of the product against the concentrations of MEP were used to find the calibration curves.

To examine the MEP content in a bulk blend and to verify the method, Paradin drug was prepared by mixing paracetamol, caffeine, and MEP. A0.01g of MEP was grinded well with 0.02 g of paracetamol and 0.02 g of caffeine.

2.3. The stoichiometric ratio of the product

Job's method of continuous variation was used to determine the stoichiometric ratio of a complex without having to isolate it from its bulk of interferences. This method measures a certain property that is relative to the complex formation such as absorbance [27]. Briefly, standard solutions with the equal mmoles of MEP and *p*- DMA were initially prepared and used to make a set of different mole fractions of MEP/P-DMA with a total volume up to 25 mL. The absorption intensities were then measured at λ_{max} =565nm.

2.4. Analysis of MEP in pharmaceuticals

Paradin tablet is composed of MEP, paracetamol, and caffeine. It is no longer produced by SDI, thus, it has prepared in our lab by mixing 0.01g of meprobamate with 0.02g paracetamol and 0.02g caffeine, where the total mass was 0.05 g.

A stock solution of the medicinal mixture of Paradin was prepared by dissolving a mass of 0.01g of Paradin blend in 0.5 mL of the freshly mixed acidic mixture H_2SO_4:HCl (9.5: 0.5 v/v) and 0.5 mL of *p*-DMA was added. The absorption of the resulted solution (0.802 AU) was corresponded to 5.87×10^{-4} mol/L of the product.

3. Results and Discussion

In this study, a colorimetric method was developed and validated for the direct quantification of the banned pharmaceutical compound of MEP without any separation. MEP was converted into a colored derivative by the reaction with *p*-DMA in an acidic medium (HCl and H_2SO_4) to produce a red colored complex turned into a violet when added to a cold water.

3.1. Optimization

The following parameters were studied and optimized as a function of the absorption intensity.

3.1.1.The wavelength

A maximum of the colored solution of MEP was observed at a wavelength of around 565 nm, where the highest sensitivity for the detection can be obtained.

3.1.2.Thermal stability of MEP

The heat extent at which the MEP molecule can tolerate before getting dissociates thermally has scrutinized using a differential scanning calorimeter (DSC). Three different samples of MEP (0.02 g, 0.03 g and 0.04) mixed with 0.1 g paracetamol and 0.1 g caffeine were measured using a thermogravimetric method, up to 160 °C. The resulted thermographs showed a thermally stable MEP **Figure 1.**

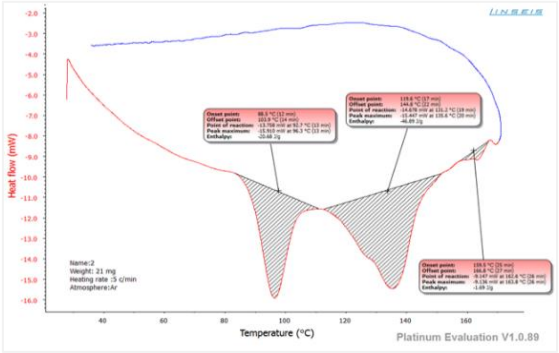

Figure 1. Thermogravimetric analysis for a mixture of MEP (0.03g), paracetamol (0.1g), and caffeine (0.1g).

3.1.3.Effect of temperature

The standard solutions of MEP were measured using a colorimetric approach at different temperatures (90-135°C). The resulted graphs exhibited no significant change of the absorbance intensity beyond 100 °C. Below 100 °C, the expected color did not appear after 25min, perhaps indicates that heating up to 100 °C is necessary to form the colored derivative. **Figure 2.** Shows that the optimum reaction temperature to form derivatized MEP was ~120 C°.

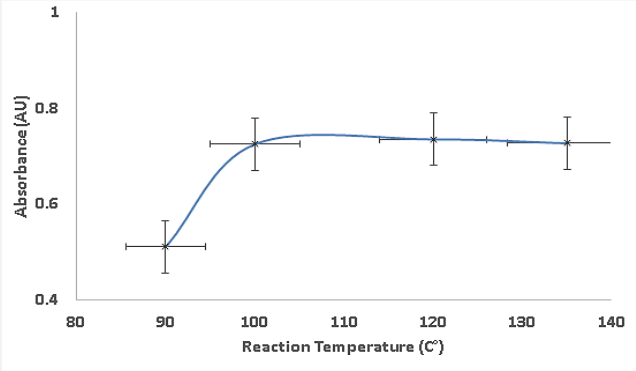

Figure 2. Effect of temperature on the colorimetric reaction of MEP.

A mechanism of the colorimetric reaction of MEP with the *p*-DMA could be suggested based on the effect of an acidic medium along with the temperature (> 100 °C), which leads to a possible rearrangement of the MEP molecule. This arranged MEP may convert into an active intermediate cation ready for the next step of coupling with an aldehyde molecule and therefore, a colored product was observed [28]. Temperature below 100 °C did not show the red colored product since the overall energy of the reaction could not meet the activation energy of the colorimetric reaction. Different reaction temperatures caused different intermediate and final products with different colors. The color of the intermediate was orange at 90 °C but turned to pinkish upon adding the ice water. Whilst, the color of the intermediate became dark red at 120 °C but turned to purple upon adding the ice water. However, when the colorimetric reaction was 135 °C; the color of the intermediate became brownish-yellow and turn to dark brownish-red upon adding the ice water.

3.1.4. Acidic hydrolysis of MEP

The proposed colorimetric derivatization of MEP involves the conversion of MEP into a cationic intermediate ready for the next step of nucleic substitution with an appropriate aldehyde to yield a colored derivative in an acid mixture. This reaction was aided using H_2SO_4 (a dehydrator) and HCl (an acidity modifier). Different molar ratios of H_2SO_4: HCl were tested to indicate the best molar ratio to form the colored product. The selected molar ratio for the acidic mixture was 19:1 V% Different compositions of the acid mixture were studied by changing the volumetric ratio of sulfuric acid and hydrochloric acid while examining their effect on the formation of the colored product. The best intensity obtained when the volume ratio of sulfuric acid was higher than 80%. Whereas a small ratio of hydrochloric acid was enough to justify the intended role. There was no dramatic outcome for the composition of the acidic mixture. Yet, the selected ratio for the acidic mixture was V% (19:1) for sulfuric acid versus hydrochloric acid. The samples were highly acidic and may corrode the inner facet of glass or quartz cuvettes, therefore plastic cuvettes were used instead as per their durability in extreme acidity and transparency in the visible area of the spectrum.

3.1.5.Effect of time

3.1.5.1.Heating time

The colorimetric reaction of MEP was examined under heating up to 140°C for 45 min time course. The progress of results was followed using thin-layer chromatography (TLC) sheets. Results showed no change in the absorption of the mixture after 25 min.

3.1.5.2.Cooling time

The effect of time after adding the product to a cold-water was studied against the absorption intensity at a wavelength of 565 nm. **Figure 3a.** Shows that the highest absorbance intensity occurred at the first 3 minutes of cooling the product. Later on, the absorbance intensity decreased dramatically, which may imply that best results could be obtained at the first 3 minutes directly after the addition to the cold distilled water. The highest absorption intensity was observed at 3:4 volume ratio of cold water: sample, see **Figure 3b.**

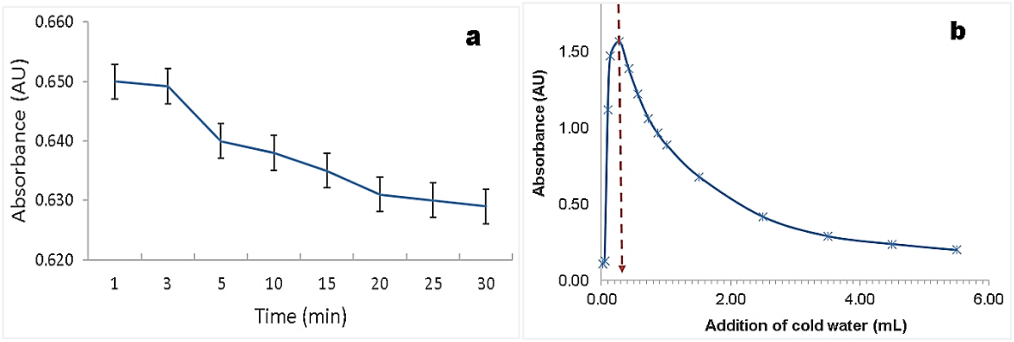

Figure 3. Effect of time and volume of the added cold water on the absorption intensity of the colored product.

3.2. The calibration curves

Figure 4 (a, b). Shows the calibration curves using a range of concentrations (1.97×10^{-6} - 1.35×10^{-3} mol/L) of the MEP standard solutions. Two major slopes covering the whole range starting from 1.23×10^{-3} mol/l down to the concentration of 1.97×10^{-6} mol/l representing the limit of quantification LOQ for MEP. It was more convenient to separate the calibration curve into two ranges, the first covers the MEP concentration range of (2.35×10^{-4} - 1.35×10^{-3} mol/l) like denoted in the **Figure 4a.** While the second covers the range of trace concentration of MEP (1.97×10^{-6} – 6.36×10^{-5} mol/l), as represented in the **Figure 4 b.**

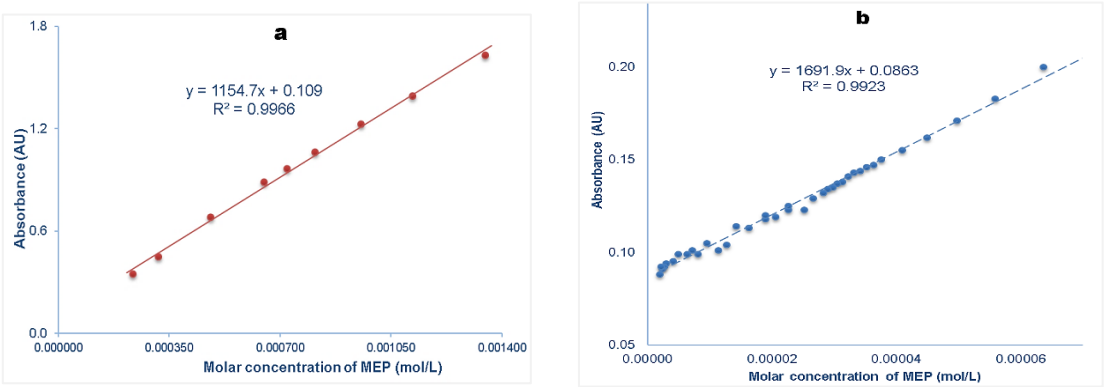

Figure 4. The calibration curves of MEP standard solutions, (a) at a range of 2.35×10^{-4} - 1.35×10^{-3} mol/L and (b) at a range of 1.97×10^{-6} – 6.36×10^{-5} mol/L.

3.3. Method validation and obedience to Beer's law

The relative standard deviation RSD has worked out using an excel function of [(STDEV(data set)/AVERAGE(data set)], which found to be 1.19% for the first calibration curve **Figure 4a.** while RSD was 1.05% for the calibration curve with least concentrations **Figure 4b.** The apparent molar absorption coefficients (Ɛ) were 1154.7 and 1691.9 $L.mol^{-1}.cm^{-1}$ respectively. The limit of detection LOD was 1.88×10^{-6} mol/L while the limit of quantification LOQ was (1.97×10^{-6} – 1.35×10^{-3} mol/L).

3.4. The stoichiometric ratio of the product

Job's method was used to determine the exact number of moles of *p*-DMA required to react colorimetrically with one mole of MEP. **Figure 5.** Shows the stoichiometry of the complex which was 1:1 mole fraction. This means, however, MEP having two functional groups of carbamates, only one have the ability to enroll in a reaction with *p*-DMA.

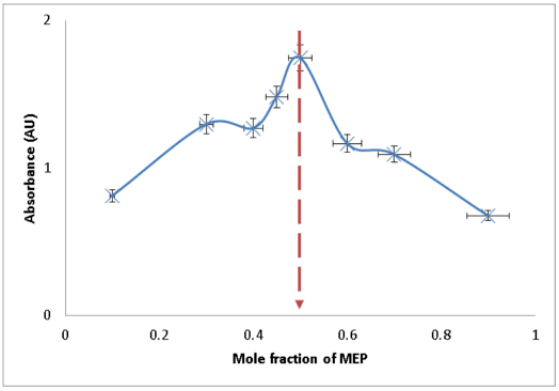

Figure 5. Job's method of continuous variation at λ_{max} =565nm, optimizing the mole ratio of (MEP/P-DMA) to (1:1).

3.5. MEP in the pharmaceutical mixture

The mass of MEP in a 0.01g portion of the drug bulk can be calculated as follows:

$W_{MEP=}$ ($W_{Portion}\times W_{MEP\ original}$) / W_{Total}= (0.01×0.01) / 0.05= 0.002g

W_{MEP} Mass of MEP in a portion of the blend bulk

$W_{MEP\ original}$ The total mass of MEP in the blend bulk

$W_{Portion}$ Mass of a portion of the blend bulk in grams

W_{Total} Total mass the blend bulk in grams

The concentration of MEP in a portion of 0.01g of the blend bulk:

C_{MEP}= ($W_{MEP(g)}\times1000$) / ($MM\times V_{Solu(mL)}$)= (0.002×1000) / (218.25×1.5)= 6.11×10^{-3} mol/l

C_{MEP} The concentration of MEP in a portion of the blend bulk

$W_{MEP(g)}$ Mass of MEP in a portion of the blend bulk in grams.

MM Molar mass of MEP

$V_{Solu(mL)}$ The total volume of the prepared solution in milliliters

The recovery% as an essential parameter for the validation issue represents a proportion of the amount of the analyte, which can be detected out of the original amount in a specimen:

Recovery= [$C_{Measured}/C_{Actual}$]×100%= [5.87×10^{-4} / 6.11×10^{-4}] ×100%= 96.07%.

C_{Actual} The actual concentration of MEP in the standard

4. Conclusion

The developed colorimetric approach used here for the analysis and detection of the MEP in some bulk pharmaceuticals shows a very high sensitivity (very low LOD value) toward the determination of MEP. In addition, there was no major interference from common medicinal ingredients. The method was simple, environmentally benign, cost-effective, did not require specific preparation, and could be applied for quantifying any carbamate-containing compounds.

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