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Quantitative Estimation of Bumadizone and Phenylbutazone in Bulk and Pharmaceutical Dosage Form Using Two Spectroscopic Methods

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Abstract

Two simple, accurate, sensitive and economic analytical techniques were carried out for quantitative estimation of Bumadizone and Phenylbutazone either in bulk or pharmaceutical preparation. The first method is derivative spectrophotometry which carried out for their quantification measurement of the two drugs in their binary mixtures, the zero crossing of the wavelengths detected, and calibration curves were depicting the linearity. The wave length were found to be 243nm and 256nm for, Bumadizone and Phenylbutazone respectively. Both of peaks were symmetrical in nature and low value of tailing when plates were scanned. Adequate separation of the two drugs enabled the development of a selective and specific method of analysis. The second method was carried out using Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopic, the first derivative of the amplitudes of bumadizone and phenylbutazone, were measured at 1179.97 cm-1 and 1092.01 cm-1 for bumadizone and phenylbutazone, respectively, and the extract was directly measured in liquid phase mode using a specific cell Znse. Concluded that these methods can be used to quantify the pure and formulations containing Bumadizone and Phenylbutazone. Moreover, validation of these methods was carried out following International Conference of Harmonisation guidelines.

***Keywords:** UV Spectrophotometry, Zero Cross, First Derivative, Bumadizone And Phenylbutazone, Ftir-Atr Spectroscopy*

Introduction

Bumadizone (BUM) chemically known as 2-[anilino(phenyl)carbamoyl] hexanoic acid (FIG.1) [1], is a non-steroidal anti-inflammatory drug; its oral preparation has been used for the treatment of rheumatic disorders and post-traumatic edema [2]. Few methods have been reported for its estimation. These methods include determination of BUM and its two reported metabolites,by HPLC [3]. A stability indicating HPLC-UV method is also reported for the determination of BUM in the presence of its alkaline degradation product, N-N diphenylhydrazine, which was prepared by refluxing with 1M NaOH for 7 hrs [4].

Phenylbutazone (PB) chemically known as 4-Butyl-1,2-diphenyl-pyrazolidine-3,5-dione, (FIG.1)[1], is a nonsteroidal anti-inflammatory drug (NSAID) effective in treating fever, pain, and inflammation in the body. As a group, NSAIDs are non-narcotic relievers of mild to moderate pain of many causes, including injury, menstrual cramps, arthritis and other musculoskeletal conditions. [5], Few methods have been reported for its estimation. These methods include determination of PB and

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oxyphenbutazone in plasma by HPLC and gas chromatography mass spectrometry [6]. the pharmaceutical analysis, has demonstrated the possibilities offered by derivative spectrophotometry. This technique has proven useful in the assay of single components in the presence of excipients [7] or degradation products, [8] and in the analysis of two-component mixtures [9]. Fourrier Transform-Infrared (FT-IR) spectroscopic analytical methods are convenient, rapid, and accurate methods, especially when in conjunction with Attenuated Total Reflectance (ATR) technology. ATR is a leading FT-IR sampling tool which typically eliminates sample preparation thus saving considerable analysis time due to its ease-of-use and speed of analysis [10]. Selection of the best; ATR crystal and accessory; further enhances FT-IR sampling success and simplifies sample handling [11]. Moreover, ATR technique provides a simple approach that control many sample handling problems and explores the mid-IR spectroscopy as an analytical technique in a number of areas, including pharmaceutical samples [12].

The aim of present study was planned to develop a method and validate the developed method for the combination of Bumadizone and Phenylbutazone in authentic and marketed sample by UV spectrophotometric and ATR-FTIR methods. Moreover, derivative spectroscopy was used for data processing to overcome the spectral overlap to allow simultaneous estimation.

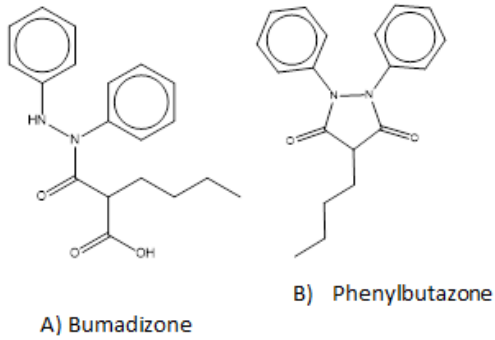

FIG.1. **Chemical structure of A); BUM, B); PB.**

Material And Methodology

Instrumentation:

Schimadzu ultraviolet/ visible recording spectrophotometer (UV-160A PC, Schimadzu, Kyoto, Japan).

The IRAffinity-1 Fourier Transform Infrared Spectrophotometer (Schimadzu Corporation, Tokyo, Japan) was connected to an ATR-8200H/8200HA base unit. A demountable liquid transmission cell (Wilmad Lab glass, Buena, NJ, USA) with ZnSe trough plate 45o prism (wave number range 10000-55-cm^{-1}, refractive index 2.4, 1 mm thick, and 0.05 mm optical path-length Transmission Range (700-4600cm^{-1})) was used. An automatic pipette (10-100 µg) was used for carrying either sample solution or standard solution into the flow cell.Ultrasonic processor, sonicator (type USR3/2 907, Julabo Labortechnik, D-7633 Seelbach, West Germany). Magnetic stirrer (Wheaton, type rc-2, Rikakikai corp,Tokyo, Japan). Schimadzu electric balance (type AGE-220, Schimadzu, Kyoto, Japan).

Reagents and Chemicals

Bumadizone (99.70 ± 1.14), was supplied by October pharma S.A.E., 6 October City, Egypt and Octomotol® tablets (Batch No. B03660613) labeled to contain 110 mg bumadizone per tablet were purchased from market.

Phenylbutazone (99.90 ±1.11), was supplied by Salutem Pharma GmbH, Berlin, Berlin, Germany , Dutsch ® tablets, labeled to contain 100 mg phenylbutazone per tablet were purchased from market. Ethanol (absolute) was supplied by Merk KGaA, Darmstadt, Germany. Chloroform (HPLC grade, for liquid sample preparation) was supplied by Sigma- Aldrich, Germany.

Standard and test Solutions

Stock standard solutions

Stock solutions (100µg/mL) from BUM and PB were prepared by dissolving 2.5 mg in 25 mL volumetric flask with 10 mL ethanol for first method and with chloroform for second method, sonication for 10 min was used to ensure complete dissolution and complete the volume with suitable solvent.

Laboratory-prepared mixtures

Different aliquots of BUM and PB equivalent to (200-800µg), were transferred into three series of 10 ml volumetric flasks. All the flasks were completed to volume with ethanol for first method and with chloroform for second method, to obtain different laboratory prepared mixtures.

Sample preparation

Twenty Octomotol® tablets® (110 mg BUM) were weighed and finely grounded. A quantity equivalent to 2.5mg BUM was quantitatively transferred into 25 ml volumetric flask, 10 ml ethanol was added and the flask was sonicated for 10 min. The flask was then completed to volume with ethanol for first method and with chloroform for second method (100µg/ml). The solution was filtered. Aliquots of this solution equivalent to (250 -750 µg), were transferred into separate 10 ml volumetric flasks and completed to volume with the suitable solvent. The same extraction procedure was applied for Dutsch ® tablets ® (100 mg PB) to prepare sample solution concentration (100µg/ml) of each pharmaceutical formulation. Aliquots of PB extracted solution equivalent to (250 - 750 µg) were transferred into 10 ml volumetric flasks and completed to volume with the suitable solvent.

Procedures and calibration curves

Selection of Analytical Wavelength

Working standard solutions of 20–80 μg/mL of each drug were prepared in a suitable solvent by appropriate dilution. For first method the spectrum was recorded between 200 and 400 nm, and all zero-order spectrums (D_0) were converted to first derivative spectrums (D1) using delta lambda 2 and scaling factor 7. The overlain first derivative spectrums of BUM and PB at different concentrations were recorded. The zero crossing point (ZCP) of BUM was found to be 243 nm, and ZCP of PB was found to be 256nm. The results are displayed in **TABLE 1**.

From second method each prepared solution, 10 µL was placed on a diamond cell ATR accessory (ZnSe) with the help of a micro pipette to record the spectrum. All spectra were collected by co-addition of 40 scans at a resolution of 5 cm-1 in the range of 4000-500 at 1.93 cm^{-1} data spacing. The spectrum of each standard or sample was subjected to the ratio of that to a fresh background spectrum recorded from the uncovered removable diamond crystal. All analyses were carried out at room temperature, and the spectra were each recorded and auto smoothed to remove noise. ATR crystal was carefully cleaned with a cellulose tissue soaked in acetone after each sample to remove any residue.

Construction of calibration curves

Aliquots of BUM stock and PB solution equivalent to 200-900 µg and 200-800µg respectively, were accurately transferred into a series of 10 ml volumetric flasks and the volumes were completed with ethanol. The first derivative spectra were recorded, the wavelength were measured at 243nm and 256nm of BUM and PH respectivly (zero crossing points). Aliquots of BUM and PB stock solutions, equivalent to 200-800 µg and 200-800 µg respectively, were accurately transferred into two series of 10 ml volumetric flasks and the volumes were completed with chloroform. The first derivative spectra were recorded, the peak amplitude were measured at 1179.97 $cm^{-1}$and 1092.01 cm^{-1} of BUM and PH respectively (zero crossing points).

Method Validation [13]

The proposed method was validated in terms of linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ), and reproducibility. The accuracy was expressed in terms of percent recovery of the known amount of the standard drugs added to the known amount of the pharmaceutical dosage forms. The precision (% relative standard deviation-% RSD) was expressed with respect to the repeatability, intraday, and interday variation in the expected drug concentrations. After validation, the developed

methods have been applied to pharmaceutical dosage form.

Linearity

Standard samples of BUM and PB were prepared in different concentration range. Each concentration was analyzed three times. Good linearity of the calibration curves was verified by the high correlation coefficient. The analytical data of the calibration curve including standard deviations for the slope and intercept (Sb, Sa) are summarized in **(TABLE1). (FIG.2), (FIG.3).**

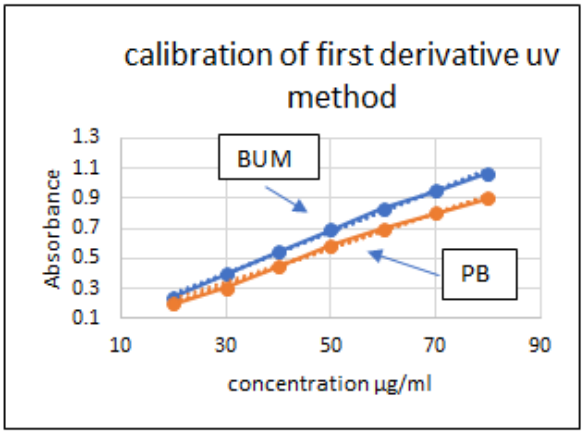

FIG.2. **Linearity Curve of BUM and PB using D1 Method At Range (20-80µG/ML)**

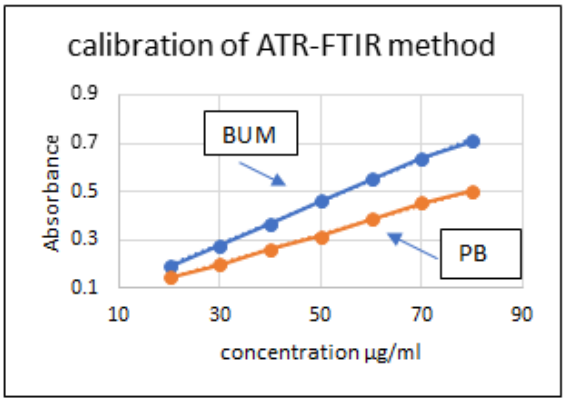

FIG.3. **Linearity Curve of BUM and PB using ATR-FTIR Method At Range (20-80µG/ML).**

Accuracy

Accuracy was assessed by the determination of the recovery of the method by addition of standard drug to the pre quantified sample preparation at 3 different concentration levels 80, 100, and 120%, taking into consideration percentage purity of added bulk drug samples. Each concentration was analyzed 3 times, and average recoveries were measured. The mean percentage recoveries are displayed in **(TABLE.1)**.

Precision

The repeatability was evaluated by assaying 6 times the sample solution prepared for assay determination. The intraday and interday precision study of BUM and PB was carried out by estimating different concentrations of BUM and PB (40, 50, and 60 μg/mL), 3 times on the same day and on 3 different days (first, second, third) and the results are reported in terms of % RSD, the data are displayed in (**TABLE.1**).

Reproducibility

The absorbance readings were measured at a different laboratory for sample solution using

another spectrophotometer by another analyst, and the values obtained were evaluated using t-test to verify their reproducibility.

Detection Limit and Quantitation Limit

ICH guideline describes several approaches to determine the detection and quantitation limits. These include visual evaluation, signal to-noise ratio, and the use of standard deviation of the response and the slope of the calibration curve. In the present study, the LOD and LOQ were based on the third approach and were calculated according to the $3.3\sigma/S$ and $10\ \sigma/S$ criterions, respectively, where σ is the standard deviation of the y-intercepts of the regression lines and S is the slope of the calibration curve, the data are displayed in (**TABLE.1**).

Results and Discussion

For first method **(FIG.4)** shows the absorption (zero-order) spectra of bumadizone and phenylbutazone. The large overlap of the spectral bands of the components at 200-300 nm prevents the formation from the total zero-order spectrum of any spectral future that could be used for analytical purposes. The first derivative spectra allowed the determination of bumadizone in presence of phenylbutazone. **(FIG.5)** shows the first derivative spectra of bumadizone and phenylbutazone; The overlain first-order derivative spectrum of bumadizone and phenylbutazone at different concentrations revealed that at 256 nm a different concentration of bumadizone possesses zero D1 absorbance, whereas phenylbutazone possesses significant D1 absorbance. In a similar manner, at 243nm, different concentrations of phenylbutazone possess zero D1 absorbance, whereas bumadizone possesses significant D1 absorbance. Considering the above facts, wavelengths 256 nm and 243 nm were selected for the estimation of phenylbutazone and bumadizone respectively. The proposed method has been extensively validated as per ICH guidelines. Summary of validation parameters for the proposed method is given in **(TABLE.1)**. Linearity was assessed for phenylbutazone and bumadizone by plotting calibration curves of the D_1 absorbance versus the concentration over the concentration range 20-80 μg/mL. **(FIG.6)** The correlation coefficients (R^2) for phenylbutazone and bumadizone were found to be 0.9974 and 0.9962, respectively **(TABLE.1)**. The following equations for straight line were obtained for bumadizone and phenylbutazone.

linear equations:

BUM, y =0.012x - 0.0363,

PB, y = 0.012x - 0.0363.

The main benefit of using a diamond cell ATR smart accessory in second method is its simplicity in handling. It only requires to place the sample on the crystal and scanned against the background of the clean crystal. Zero order spectra of BUM and PB (**FIG.7**) showed severe overlap, moreover in dosage form, which prevents their direct measurement without preliminary separation. Thus, the derivative of the amplitude spectra was suggested to resolve this problem. The first derivative spectra (**FIG.6**) of BUM showed a peak at 1179.97 cm^{-1} where PH displayed zero value and PH showed a peak at 1092.01 cm^{-1} where BUM displayed zero value. The above selected wavenumbers absorbances or amplitudes were used successfully for the determination of the aforementioned drugs without any interference. The correlation coefficients (R^2) for bumadizone and phenylbutazone were found to be 0.9989and 0.9984, respectively (**TABLE.1**). The following equations for straight line were obtained for bumadizone and phenylbutazone.

linear equations: (**FIG.7**).

BUM, y = 0.012x -0.0363,

PB, y = = 0.0061x + 0.0181.

The above selected wavenumbers absorbances or amplitudes were used successfully for the determination of the aforementioned dosage form without any interference.

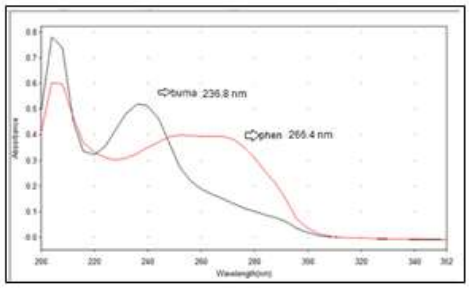

FIG.4. **D_0 spectrum of BUM at 236.8nm (55 µg/mL) and D_0 spectrum of PB at 266.4nm (55 µg/mL).**

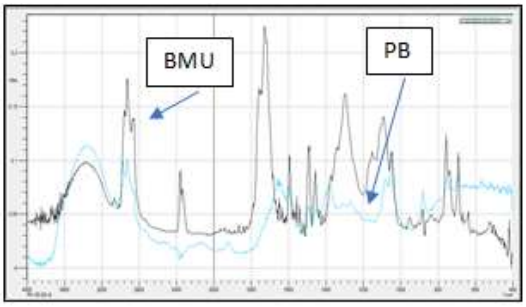

FIG.5. **ATR-FTIR spectrum of BUM (45 µg/mL) and of PB (45 µg/mL).**

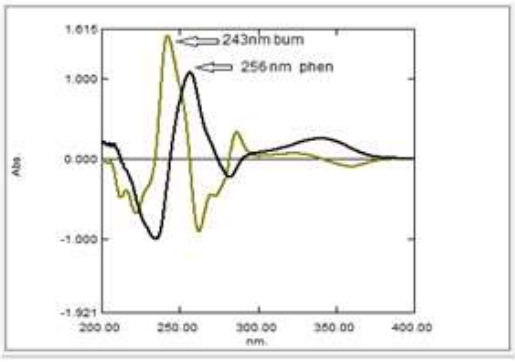

FIG.6. **D1 spectrum of BUM at 243nm (55 µg/mL) and D1 spectrum of PB at 256nm (55 µg/mL).**

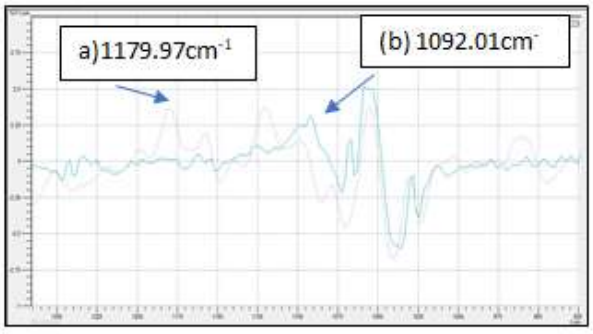

FIG.7. **First derivative ATR-FTIR spectra of a); BUM at 1179.97 cm-1 (45 µg/mL) and b); PBat 1092.01 cm-1 (45 µg/mL).**

TABLE.1. **Validation data for derivative UV spectrometric analysis.**

Method	**Derivative UV spectrometric**		**FTIR spectrometric**	
NSAIDs	BUM	PB	BUM	PB

Wavenumber of measurement (cm–1)			1078.59	2160.32
Wavelength (nm)	243	256		
Solvent	Ethanol		chloroform	
Linearity Range (µg/mL)	20-80	20-80	20-80	20-80
Regression coefficient (r2)	0.9974	0.9962	0.9989	0.9984
Slope	0.0139	0.012	0.0088	0.0061
Intercept	-0.0191	-0.0363	0.017	0.0181
LOD (µg/mL)	1.523	2.568	2.826	2.773
LOQ (µg/mL)	4.614	7.782	8.566	8.402
Sb	0.003	0.007	0.004	0.007
Sa	0.0001	0.0001	0.0001	0.0001
Confidence limit of the slope	0.0001	0.0002	0.0002	0.0002
Confidence limit of the intercept	0.008	0.019	0.013	0.002
Standard error of the estimation	0.003	0.007	0.007	0.007
Inter-day (%R.S.D.)	0.165	0.152	0.257	0.288
Intra-day (%R.S.D.)	0.214	0.153	0.201	0.218
Drug in bulk	99.78±0.704	99.89±0.314	99.12±0.340	99.72±0.545
Drug in dosage form	100.02±0.449	99.92±0.243	99.72±0.442	99.83±0.664
Drug added	99.34±0.586	99.89±0.633	98.92±0.536	99.62±0.766

Conclusion

Two new precise and accurate quantitative methods, derivative UV spectrophotometry and ATR-FTIR with minimal sample preparation have been realized for the determination of bumadiazone and phenylbutazone. The zero order of UV-spectrophotometry method appeared to be unsuitable for the estimation of bumadiazone and phenylbutazone, However, the first-order derivative UV-spectrophotometry method can be used to measure the two drugs with sufficient accuracy and precision. The proposed first-order derivative spectrophotometry method is simple, rapid, and economical for routine analysis of bumadizone and phenylbutazone. The aim for using “Zero-crossing” first derivative spectrophotometry because the derivative spectrophotometric technique enhances the detectability of the minor features of the UV absorption spectrum, the first derivative spectra of both bumadizone and phenylbutazone displays features which may permit more specific and selective determination. The ATR-FTIR analysis was easily carried out for these NSAIDs in their liquid form without preliminary purification. These analytical methods are alternative to those that employ separation techniques. This can significantly reduce both expensive laboratory analysis and chemical waste.

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