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ORIGINAL ARTICLE

Utilization of bromination reactions for the determination of carbamazepine using bromate–bromide mixture as a green brominating agent

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Pharmaceutical analysis

Abstract One titrimetric and two spectrophotometric procedures have been developed for the assay of carbamazepine (CBZ) in bulk drug, formulations and spiked human urine. The methods are based on the bromination of CBZ by the bromine generated *in situ* by the action of the acid on the bromate–bromide mixture. The twin advantages of avoiding liquid bromine and analysis in a cost-effective manner are realized. In titrimetry, the drug was treated with a known excess of bromate–bromide mixture in hydrochloric acid medium followed by the determination of unreacted bromine iodometrically. Spectrophotometry involves the addition of a measured excess of bromate–bromide reagent in acid medium to CBZ, and after the reaction is ensured to be complete, the residual bromine was determined by reacting with a fixed amount of either methyl orange and measuring the absorbance at 510 nm (method A) or indigo carmine and measuring the absorbance at 610 nm (method B). Titrimetric procedure is applicable over the range of 1.00–7.50 mg CBZ, and the calculations are based on a 1:1 reaction stoichiometry (CBZ:$KBrO_3$). In spectrophotometric methods, Beer's law is valid within concentration ranges of 0.25–1.50 and 0.50–6.00 μg ml^{-1} CBZ for methods A and B, respectively. The proposed methods were successfully applied to the determination of CBZ in tablets and syrup, in addition to spiked human urine by the spectrophotometric methods, with mean recoveries of 95.50–104.0% and the results were statistically compared with those of an official method by applying Student's *t*-test and *F*-test.

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1. Introduction

Carbamazepine (CBZ), chemically known as 5H-dibenz-[b,f]-azepine-5-carboxamide (Merck Index, 2006), is an anticonvulsant and mood stabilizing drug used primarily in the treatment of epilepsy and bipolar disorder, as well as trigeminal neuralgia. The drug is official in the British Pharmacopoeia (1973) which describes a UV-spectrophotometric method for its assay in tablets. Since its introduction into clinical medicine in the mid-1960s, literature on the methods for the determination of CBZ in biological

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materials is vast (> 120 published articles). In contrast, a limited number of techniques have been reported for the assay of CBZ in pharmaceutical dosage forms and include HPLC (Panchagnula et al., 1998; Tatar Ulu, 2006; Demirkaya and Kadioglu, 2005, 2008; Yuan et al., 2003), LC (Predrag et al., 2009; Walker, 1988), GC (Kadioglu and Demirkaya, 2007; Liu et al., 1991), flow injection-spectrophotometry (Çomoğlu et al., 2006), FI-spectrofluorimetry (Huang et al., 2002), chemiluminescence (CL) (Lee et al., 2003), FI-CL (Xiong et al., 2009), electrolysis-fluorescence (Pan and Yao, 1998), polarography (Pachecka and Giovanoli, 1982), UV-spectrophotometry (Tatar Ulu, 2006; Demirkaya and Kadioglu, 2008) and visible spectrophotometry (Rao and Murty, 1982; Agrawal et al., 1989). All these methods are reviewed in Tables 1 and 2.

In spite of the suitability of the proposed methods, many require expensive instruments or materials such as chromatographic, flow injection and chemiluminescence techniques. Besides, chromatographic methods (Panchagnula et al., 1998; Tatar Ulu, 2006; Yuan et al., 2003; Predrag et al., 2009; Walker, 1988; Kadioglu and Demirkaya, 2007; Liu et al., 1991) need a suitable compound as internal standard, which makes the procedure more complex. Visible spectrophotometry, because of its simplicity and cost effectiveness, sensitivity and selectivity, fair accuracy, precision and easy access in most quality control laboratories, has remained competitive in an area of chromatographic techniques for pharmaceutical analysis.

To the best of our knowledge, no titrimetric method has ever been reported and there are only two reports on the use of visible spectrophotometry for the determination of CBZ in pharmaceuticals. The first report (Rao and Murty, 1982) is based on the oxidation of CBZ with sodium metaperiodate in acidic medium after heating for 1 h, extraction of the chromogen into *n*-butanol before measuring the absorbance at 410 nm. The second method (Agrawal et al., 1989) is based on the reaction of amide group in CBZ with hydroxylammonium chloride–NaOH under hot conditions, followed by reaction with ferric chloride in HCl medium and measuring the absorbance at 510 nm. The visible spectrophotometric methods currently available (Rao and Murty, 1982; Agrawal et al., 1989) involve extraction or heating step and their sensitivity is poor.

The scientific references found in the CAS and SCI database, relating to green analytical chemistry or environmental-friendly analytical methods have been growing significantly in recent years (Armenta et al., 2008). The recent development of new analytical methods with good characteristics such as selectivity and sensitivity is not sufficient; modern analytical methods need to be green (Sharma et al., 2003; Vidotti et al., 2004). Hence, the purpose of this study was to develop three new methods for the determination of CBZ based on bromination of CBZ by a green brominating agent (i.e. bromine-generated *in situ*). The methods employ titrimetric and spectrophotometric techniques, and use bromate–bromide mixture, methyl orange (MO) and indigo carmine (IC) as reagents. The proposed methods have the advantage of accuracy and precision besides being free from interference from common tablet excipients.

2. Experimental

2.1. Apparatus

All the absorbance spectral measurements were made using a Systronics model 106 digital spectrophotometer equipped with 1 cm matched quartz cells.

2.2. Materials and reagents

Pharmaceutical grade carbamazepine (CBZ) was received from Jubilant Organosys Ltd., Mysore, India, as a gift and used as received. All pharmaceutical preparations were obtained from commercial sources in the local market. All reagents and chemicals used were of analytical reagent grade and distilled water was used throughout the study.

A standard stock solution of bromate–bromide mixture equivalent to 5 mM $KBrO_3$ and 10-fold molar excess of KBr was prepared by dissolving accurately weighed 0.209 g of potassium bromate (s.d. fine-chem Ltd., Mumbai, India) and 1.488 g of potassium bromide (Merck, Mumbai, India) in water and diluting to volume in a 250 ml calibrated flask, and used in titrimetric method. Another stock standard solution of $KBrO_3$–KBr equivalent to 200 μg ml^{-1} $KBrO_3$

Table 1 Chromatographic methods reported for the determination of CBZ in pharmaceuticals.

Technique	Chromatographic conditions			LOD (μg ml^{-1})	Rang (μg ml^{-1})	References
	Mobile phase	Flow rate (ml min^{-1})	Detection			
HPLC	0.01 M potassium phosphate buffer of pH 7/acetonitrile/methanol (11:5:3)	1.0	At 214 nm	0.100		Panchagnula et al. (1998)
HPLC	Acetonitrile:water (75:25, v/v)	1.0	At 285 nm	0.055	0.2–2.0	Tatar Ulu (2006)
HPLC	Acetonitrile–Milli-Q grade water (30:70, v/v)	1.0	UV at 220 nm	0.05	0.25–25	Demirkaya and Kadioglu (2005)
HPLC	(28:72, v/v) acetonitrile:0.02 M sodium phosphate buffer (pH 7.8)	1.0	UV at 230 nm	0.018	5.0–25.0	Yuan et al. (2003)
HPLC–DAD	Acetonitrile–Milli-Q grade water (30:70, v/v)	1.0	At 220 nm	0.05	0.25–25	Demirkaya and Kadioglu (2008)
LC	(50:50, v/v) methanol–10 mM ammonium acetate buffer, pH adjusted to 2.21 with glacial acetic acid	1.5	UV at 260 nm	0.0125	100–500	Predrag et al. (2009)
LC	THF–methanol–water (8:37:55)	1.0	At 254 nm		0.2–1.7	Walker (1988)
GC			FID		2–30	Kadioglu and Demirkaya (2007)
GC	Carrier gas is nitrogen	20	FID			Liu et al. (1991)

Table 2 Non-chromatographic techniques reported for the determination of CBZ in pharmaceuticals.

Technique	Method	λ_{max}	LOD	Range	References
UV-spectrophotometry	The "peak-to-peak amplitudes" was measured at the respected wavelength	270.6/282.6	1.25 μg ml^{-1}	4.0–10 μg ml^{-1}	Tatar Ulu (2006)
First order-derivative UV-spectrophotometry	The "peak-to-peak amplitudes" was measured at the respected wavelengths	285 nm	0.25 μg ml^{-1}	1.25–25 μg ml^{-1}	Demirkaya and Kadioglu (2008)
Second order-derivative UV-spectrophotometry		287 nm	0.25 μg ml^{-1}	1.25–25 μg ml^{-1}	
FI spectrophotometry	The carrier solvent was 1% SDS, and certain FIA parameters were examined to find the optimum conditions, such as wavelength of detection, flow rate, and injection volume	288 nm	8.34×10^{-7} M	1.08×10^{-5}–6.48×10^{-5} M	Çomoğlu et al. (2006)
FI photochemical spectrofluorimetry	CBZ was converted to a strongly fluorescent compound upon on-line photochemical reaction in a dilute HCl	λ_{em} = 478 nm λ_{ex} = 254 nm	0.08 ng ml^{-1}	2–250 ng ml^{-1}	Huang et al. (2002)
Chemiluminescence (CL)	Based on the chemiluminescence produced in the reaction of tris(2,2′-bipyridine) ruthenium(III) and CBZ in an acidic medium	λ_{max} = 675 nm	2.5×10^{-7} mol l^{-1}	4.0×10^{-3}–8.6×10^{-7} mol l^{-1}	Lee et al. (2003)
FI-CL	Based on that CBZ could significantly enhance the weak chemiluminescence (CL) of the reaction of potassium permanganate and sodium sulfite		2.0×10^{-10} g ml^{-1}	1.0×10^{-9}–1.0×10^{-6} g ml^{-1}	Xiong et al. (2009)
Electrolysis-fluorescence	CBZ was treated with Mn(II), H_2SO_4 and anhy. ethanol. After electrolysis with a Pt-wire electrode by applying a voltage of 1.2 V, the fluorescence intensity due to oxidation of CBZ was measured	Fluorescence intensity measured at 478 nm, and excitation at 254 nm	0.8 nM	8.4 nM–1.76 μM	Pan and Yao (1998)
Polarography					Pachecka and Giovanoli (1982)
Visible spectrophotometry	Based on the oxidation of CBZ with sodium metaperiodate in acidic medium, then extraction the chromogen into n-butanol	410 nm	NA	10–100 μg ml^{-1}	Rao and Murty (1982)
Visible spectrophotometry	Based on the reaction of amide group in CBZ with hydroxylammonium chloride, followed by reaction with ferric chloride in HCl medium	510 nm		4–80 μg ml^{-1}	Agrawal et al. (1989)
Visible spectrophotometry	Based on the reaction of CBZ with a measured excess of bromate–bromide mixture in acid medium and determining the residual bromine by				Present methods
	(a) reacting with a fixed amount of methyl orange and measuring the absorbance at 510 nm	510 nm	0.05 μg ml^{-1}	0.25–1.50 μg ml^{-1}	
	(b) reacting with a fixed amount of indigo carmine and measuring the absorbance at 610 nm	610 nm	0.14 μg ml^{-1}	0.50–6.00 μg ml^{-1}	

was prepared in a 100 ml calibrated flask and diluted with water to get bromate–bromide solutions containing 12.5 and 50 μg ml^{-1} in $KBrO_3$ for use in spectrophotometric method A and method B, respectively.

Solutions of 5 M hydrochloric acid (Merck, Mumbai, India; sp. gr. 1.18), 5% potassium iodide (Merck, Mumbai, India), 0.03 M sodium thiosulphate (Sisco-chem Industries, Mumbai, India, assay 98%), 1% starch indicator GR (LOBA Chemie Pvt. Ltd., Mumbai, India), 60 μg ml^{-1} methyl orange (s.d. fine-chem Ltd., Mumbai, India) for method A and 200 μg ml^{-1} indigo carmine for method B (LOBA Chemie Pvt. Ltd., Mumbai, India) were prepared in water.

A stock standard solution equivalent to 1.0 mg ml^{-1} of CBZ was prepared by dissolving accurately weighed 100 mg of pure drug in 40 ml methanol and diluted to the mark in a 100 ml calibrated flask with water. This solution was used in titrimetric work and diluted appropriately with water to get the working concentrations of 5.0 and 20 μg ml^{-1} CBZ for use in spectrophotometric method A and method B, respectively.

2.3. Assay procedures

2.3.1. Titrimetry

A 1.0–7.5 ml aliquot of standard CBZ solution containing 1.00–7.50 mg of CBZ was measured accurately, transferred into a 100 ml Erlenmeyer flask and the total volume was made to 10 ml with water. The solution was acidified by adding 5 ml of 5 M HCl followed by the addition of 10 ml of bromate–bromide mixture 5 mM in $KBrO_3$ by means of a pipette. The content was mixed well and the flask was kept aside for 10 min with occasional swirling. Then, 5 ml of 5% potassium iodide was added to the flask and the liberated iodine was titrated with 0.03 M sodium thiosulphate to a starch end point. A blank titration was performed taking 10 ml of water–methanol (3:2) mixture in place of drug solution and the drug content in the aliquot was calculated from a knowledge of the amount of $KBrO_3$ reacted.

2.3.2. Spectrophotometry using methyl orange (method A)

Different aliquots (0.0, 0.5, 1.0, 1.5, ..., 3.0 ml) of a standard CBZ (5 μg ml^{-1}) solution were accurately transferred into a series of 10 ml calibrated flasks and the total volume was adjusted to 3.0 ml by adding adequate quantity of water. To each flask, 2 ml of 5 M HCl was added followed by 1.0 ml of bromate–bromide (12.5 μg ml^{-1} in $KBrO_3$). The flasks were stoppered, content mixed and allowed to stand for 15 min with occasional shaking. Then, 1.0 ml of methyl orange solution (60 μg ml^{-1}) was added to each flask, and after 5 min, the mixture was diluted to the volume with water and mixed well. The absorbance of each solution was measured at 510 nm against a reagent blank.

2.3.3. Spectrophotometry using indigo carmine (method B)

Varying aliquots (0.0, 0.25, 0.5, 1.0, ..., 3.0 ml) of a standard 20 μg ml^{-1} CBZ solution were transferred into a series of 10 ml calibrated flasks using a micro burette and the total volume was brought to 3 ml by adding water. To each flask 2 ml of 5 M HCl and 1.0 ml of bromate–bromide mixture solution (50 μg ml^{-1} in $KBrO_3$) were added. The content was mixed well and the flasks were kept aside for 15 min with intermittent shaking. Finally, 1.0 ml of indigo carmine solution (200 μg ml^{-1}) was added to each flask and the volume was adjusted to the mark with water. The absorbance of each solution was measured at 610 nm against a reagent blank.

2.3.4. Assay procedure for tablets

Ten tablets containing CBZ were accurately weighed and ground into fine powder. A portion of the powder equivalent to 100 mg of CBZ was accurately weighed into a 100 ml calibrated flask, 40 ml of methanol and 20 ml of water were added. The content was shaken for 15–20 min; the volume was diluted to the mark with water, mixed well and filtered using a Whatman No. 42 filter paper. The first 10 ml portion of the filtrate was discarded and a convenient aliquot of the subsequent portion of the filtrate was assayed by titrimetric procedure. The same tablet extract was appropriately diluted with water to get 5.0 and 20 μg ml^{-1} w.r.t. CBZ for the assay by the spectrophotometric methods A and B, respectively.

2.3.5. Assay procedure for syrup

Five milliliters of syrup (Tegrital 100 mg/5 ml) equivalent to 100 mg of CBZ into a 100 ml calibrated flask, 40 ml of methanol and 20 ml of water were added. The content was shaken for 15–20 min; the volume was diluted to the mark with water and mixed well. The resulting solution was clear and it was assayed by titrimetric procedure. The same solution was appropriately diluted with water to get 5.0 and 20 μg ml^{-1} w.r.t. CBZ for the assay by the spectrophotometric methods A and B, respectively.

2.3.6. Assay procedure for spiked human urine by the spectrophotometric methods

Five milliliters of CBZ free urine taken in a 125 ml separating funnel was spiked with 5.0 ml of a standard 200 μg ml^{-1} CBZ solution, the content was mixed well and then extracted with 3×10 ml of dichloromethane. The dichloromethane extract was collected in a beaker containing anhydrous sodium sulphate and the water-free organic layer was transferred into a 50 ml dried calibrated flask and the solvent was evaporated on a hot water bath. The residue was dissolved in 2 ml of meth-

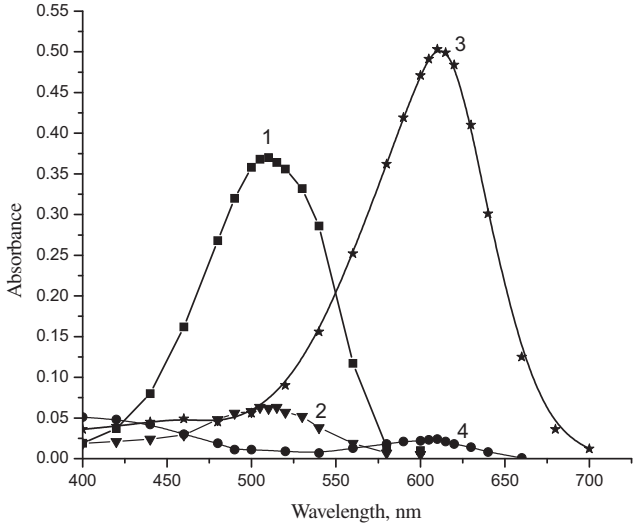


Figure 1 Absorption spectra of: (1) methyl orange in acid (2.5 μg ml^{-1} of MO); (2) blank for methyl orange; (3) indigo carmine in acid (10 μg ml^{-1} of IC); (4) blank for indigo carmine.

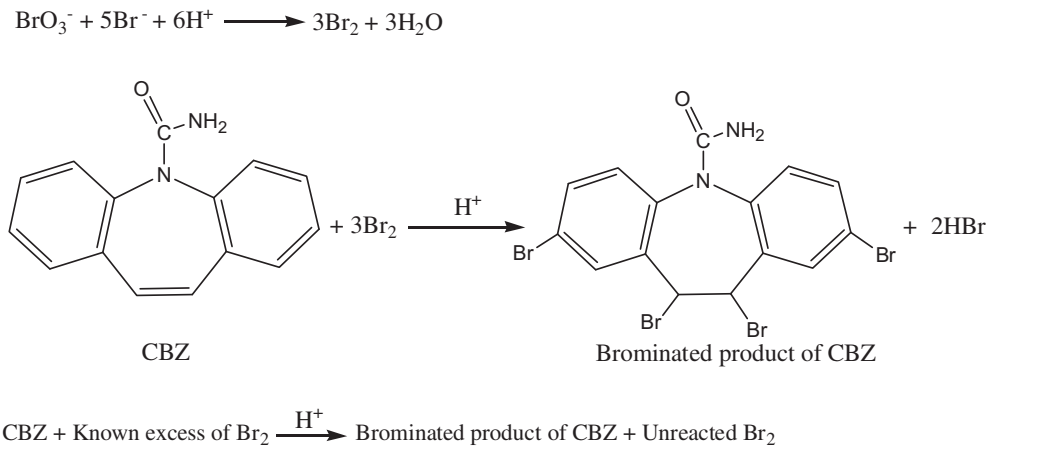
anol and the volume was diluted with water up to the mark. An aliquot of the resulting solution was analyzed using the procedures of the methods A and B.

3. Results and discussion

Carbamazepine is found to undergo bromination by bromine generated *in situ* by the action of the acid on bromate–bromide mixture. The present work deals with one titrimetric and two spectrophotometric procedures for the assay of CBZ using *in situ* generated bromine as a green brominating agent. Bromine generated *in situ* by the action of the acid on bromate–bromide mixture was achieved upon the principles of green chemistry (Anastas and Warner, 1998) such as replacement of the highly toxic and hazardous liquid bromine, no formation of hazardous byproducts, eco-friendly, easily available and monetarily cheap. The proposed methods are indirect and based on the determination of residual bromine after the reaction between the drug and bromine is judged to be complete and rely on different reaction schemes. In titrimetry, the reaction was followed by back titration of the unreacted bromine iodometrically, whereas in spectrophotometry, the reaction was followed by measuring the increase in absorbance of either methyl orange at 510 nm or indigo carmine at 610 nm (Fig. 1). The absorbance change is caused by the bleaching action of residual bromine generated *in situ* on the dyes (Fig. 2).

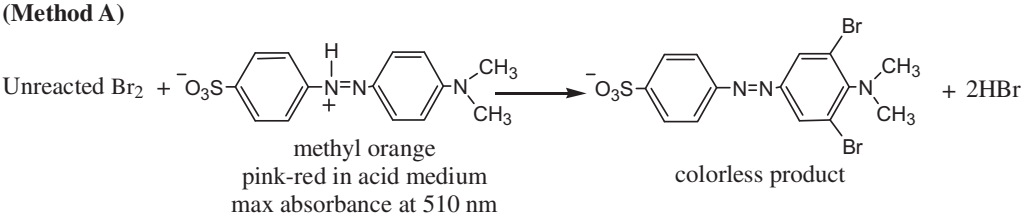
3.1. Chemistry

The reaction between carbamazepine and bromine generated *in situ* by the action of the acid on bromate–bromide mixture uses electrophilic substitution as well as addition reactions.



(Titrimetry)

(Method A)



(Method B)

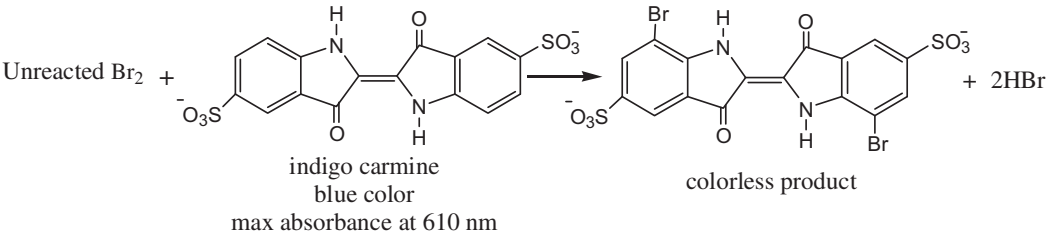


Figure 2 Tentative reaction scheme for the proposed methods.

The presence of the nitrogen atom attached to the benzene rings will direct the bromination to ortho and para positions in both the rings but the bromination occurs at the para positions only due to the steric effect of the amide which leads to a decrease in the amount of the ortho-product. The addition of the bromine will occur to the double bond at the C_3 and C_4 in the azepine ring (Fig. 2).

The reaction between methyl orange and unreacted bromine involves aromatic ring substitution as a major reaction (Laitinen and Boyer, 1972) and produces a large decrease in the molar absorptivity at respective wavelength. The most logical place for the addition of the first bromine is ortho to the N,N-dimethylamino group as this group is a strongly activating ortho and para director. Also, the addition of a second mole of bromine per mole of methyl orange must result in the bromination of another available position on one of the two rings (Fig. 2). The bromination will not occur in another ring because the protonated azo link and the sulfonate group are both deactivating meta directors for aromatic electrophilic substitution.

The reaction between indigo carmine and unreacted bromine involves the bleaching of the dye's colour by the bromination of the dye at ortho positions to –NH group of the two symmetric aromatic rings (Fig. 2).

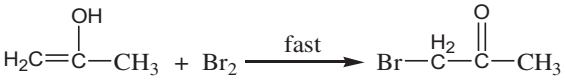
3.2. Method development

3.2.1. Selection of the solvent

Since carbamazepine is insoluble in water (Merck Index, 2006), different solvents such as ethanol, acetone and methanol were tested to prepare the stock standard solution of CBZ. Ethanol and acetone were unsuitable due to the oxidation of ethanol (Farkas et al., 1949) and bromination of acetone (Lopez-Cueto et al., 2002) by the free bromine as follows:

$$CH_3CH_2OH + Br_2 \xrightarrow{k_I} CH_3CHO + 2HBr$$

$$CH_3CH_2OH + Br_2 + H_2O \xrightarrow{k_{II}} CH_3COOH + 2HBr$$



Among the tested solvents, methanol was found to be an ideal solvent for the preparation of the standard solution of CBZ, and the minimum ratio to get a stable solution was 2:3 (methanol:water) for 1.0 mg ml^{-1} CBZ.

3.2.2. Optimization of reaction conditions

The reaction conditions as well as the various experimental variables providing accurate and precise results, and affecting the colour development of the measured species were carefully optimized.

3.2.2.1. Titrimetry. The proposed titrimetric procedure is based on the bromination reaction between CBZ and bromine generated *in situ*. A (1.00–7.50) mg of CBZ were treated with known excess of bromate–bromide mixture in acid medium, and back titrating the residual bromine iodometrically after ensuring the completion of the reaction. Hydrochloric acid medium was found to be an ideal and the reaction stoichiometry was unaffected when 2–8 ml of 5 M HCl was used in a total volume of 25 ml. Hence, 5 ml of 5 M HCl was used in the titrimetric study. The reaction was found to be complete in 10 min and contact time up to 25 min had no effect on the stoichiometry or the results but after 30 min contact time, small brown particles were developed due to the formation of brominated product of CBZ. Ten milliliters volume of 5 mM $KBrO_3$–50 mM KBr was found adequate for a quantitative bromination of CBZ in the range investigated.

3.2.2.2. Spectrophotometry. The proposed spectrophotometric methods are based on the bromination of CBZ by a measured excess of bromate–bromide mixture in HCl medium. After a predetermined time, the unreacted bromine was determined by treating it with a fixed amount of either methyl orange or indigo carmine dye, and measuring the absorbance either at 510 or 610 nm. The absorbance was found to be linearly dependent on the CBZ concentration. CBZ, when added in increasing concentrations to a fixed concentration of *in situ* generated bromine, consumes the latter and there will be a concomitant decrease in the concentration of bromine. When a fixed concentration of either dye is added to decreasing concentrations of bromine, a concomitant increase in the dye concentration results. Consequently, a proportional increase in the absorbance at the respective λ_{max} is observed with increasing the concentration of CBZ.

3.2.2.3. Optimization of the concentration of the dyes and bromate–bromide mixture. Various parameters associated with the bromination of CBZ, and subsequent destruction of the dyes by the residual bromine were optimized. Preliminary experiments were performed to fix the upper concentrations of the two dyes that could be determined spectrophotometrically in acid medium, and these were found to be 6 and 20 μg ml^{-1} for methyl orange and indigo carmine, respectively. A bromate concentration of 1.25 μg ml^{-1} in the presence of a large excess of bromide was found to bleach the pink-red colour of 6.0 μg ml^{-1} methyl orange, and the blue colour due to 20 μg ml^{-1} indigo carmine was completely destroyed by 5.0 μg ml^{-1} bromate in the presence of excess bromide. Hence, different concentrations of CBZ were reacted with 1.0 ml of 12.5 μg ml^{-1} bromate in method A and 1.0 ml of 50.0 μg ml^{-1} bromate in method B in acid medium and in the presence of a large excess of bromide, followed by determination of the residual bromine as described under the respective procedures.

3.2.2.4. Effect of acid and reaction time. Hydrochloric acid was found to be a convenient medium for the two steps involved in both methods and the absorbance of the dyes was not affected in 0.50–1.25 M HCl. However, since 1.0 M acid concentration (2 ml of 5 M HCl in a total volume of 10 ml) was found to be optimum for the bromination reaction in a reasonable time of 15 min in both methods, the same concentration was maintained for the determination of the unreacted bromine with the dyes; and even this reaction time is not critical. A 5 min standing time was found to be necessary for the complete bleaching of the methyl orange colour by the residual bromine, whereas the complete bleaching of the indigo carmine colour was instantaneous.

3.2.3. Colour stability

The absorbance of the measured colour was constant for more than 24 and 12 h for methods A and B, respectively, even in the presence of the reaction products.

3.3. Method validation

3.3.1. Quantitative data

The titrimetric procedure is applicable over the range of 1.00–7.50 mg of CBZ and the reaction stoichiometry was calculated to be (1:1) for the reaction between CBZ and $KBrO_3$.

In spectrophotometric procedures, the Beer's law was obeyed over the concentration ranges of 0.25–1.50 and 0.50–6.00 μg ml^{-1} of CBZ for methods A and B, respectively. The linear plots gave the following regression equations:

$$A = 0.0123 + 0.4953C \quad \text{for method A}$$

$$A = -0.0031 + 0.1263C \quad \text{for method B}$$

where A is the absorbance and C is the concentration in μg ml^{-1}. The correlation coefficient (r) of the calibration plots of the methods A and B is calculated to be 0.9980 and 0.9997, respectively, confirming a linear increase in the absorbance with increasing the concentration of the drug. The calculated molar absorptivities are 1.21×10^5 and 2.91×10^4 l mol^{-1} cm^{-1} for method A and method B, respectively with corresponding Sandell sensitivity values of 0.0020 and 0.0081 μg cm^{-2}. The limits of detection (LOD) and limits of quantification (LOQ) for the proposed methods were calculated using the following equations (ICH, 2005):

$$\text{LOD} = \frac{3.3 \times \sigma}{S}$$

$$\text{LOQ} = \frac{10 \times \sigma}{S}$$

where σ is the standard deviation of replicate determination values under the same conditions as for the sample analysis

Table 3 Intra-day and inter-day precision and accuracy studies.

Method[a]	CBZ taken	Intra-day (n = 7)			Inter-day (n = 5)		
		CBZ found[b]	Precision[c]	Accuracy[d]	CBZ found[b]	Precision[c]	Accuracy[d]
Titrimetry	2.0	2.05	1.47	2.5	2.04	1.62	2.00
	4.0	4.04	1.08	1.00	4.06	1.17	1.50
	6.0	5.95	1.21	0.83	6.08	1.53	1.33
Spectrophotometric (method A)	0.5	0.51	2.46	2.00	0.49	2.38	2.00
	1.0	1.03	1.85	3.00	1.01	1.92	1.00
	1.5	1.47	1.15	2.00	1.54	1.27	2.67
Spectrophotometric (method B)	2.0	1.98	2.36	1.00	2.03	2.01	1.50
	3.0	2.99	2.21	0.33	3.02	1.87	0.67
	4.0	4.06	1.31	1.50	4.07	1.50	1.75

[a] CBZ taken/found in titrimetric method is in mg and the same in spectrophotometric methods are in μg ml^{-1}.
[b] Mean value of five determinations.
[c] Relative standard deviation (%).
[d] Bias (%) = [(found − taken)/taken] × 100.

Table 4 Results of assay of CBZ formulations using the proposed methods and statistical evaluation.

Brand name	Nominal amount	Found (% of nominal amount ± SD)[a]			
		Official method	Proposed methods		
			Titrimetric method	Spectrophotometric (method A)	Spectrophotometric (method B)
Zeptol tablets[b]	100 mg	98.36 ± 1.28	97.50 ± 2.45 t = 0.73 F = 3.66	100.4 ± 1.81 t = 2.09 F = 2.00	99.12 ± 1.92 t = 0.75 F = 2.25
Tegrital syrup[c]	100 mg/5 ml	96.72 ± 1.34	95.01 ± 2.06 t = 1.59 F = 2.36	96.07 ± 2.24 t = 0.57 F = 2.79	97.23 ± 1.76 t = 0.52 F = 1.72

Tabulated t-value at the 95% confidence level is 2.78; tabulated F-value at the 95% confidence level is 6.39.
[a] Mean value of five determinations.
[b] Marketed by Sun Pharmasikkim, ranipool, India.
[c] Marketed by Piramal Healthcare Limited, Mahad, India.

Table 5 Determination of CBZ in spiked human urine sample.

Method	Spiked concentration (μg ml^{-1})	Found[a] ± SD	% Recovery ± RSD
Spectrophotometric (method A)	1.00	0.93 ± 0.02	93.16 ± 2.15
Spectrophotometric (method B)	4.00	3.61 ± 0.07	90.12 ± 1.94

[a] Mean value of five determinations.

in the absence of the analyte and S is the sensitivity, namely the slope of the calibration graph. In accordance with the above equations, the LOD values are 0.05 and 0.14 μg ml^{-1} for methods A and B, respectively with corresponding LOQ values of 0.15 and 0.42 μg ml^{-1}.

3.3.2. Accuracy and precision

To evaluate the accuracy and precision of the methods, pure drug solution at three different levels (within the working limits) was analyzed, each determination being repeated seven times. The relative error (%) and relative standard deviation (%) were less than 3.0 and indicate the high accuracy and precision for the methods (Table 3). For a better picture of reproducibility on a day-to-day basis, a series of experiments were performed in which standard drug solution at three different levels was determined each day for 5 days with all solutions being prepared afresh each day. The day-to-day relative standard deviation values were in the range of 1.17–2.38% and represent the best appraisal of the methods in routine use. These results are also given in Table 3.

3.3.3. Selectivity

In order to evaluate the selectivity of the proposed methods for the analysis of pharmaceutical formulations, the effect of the presence of common excipients, such as talc, starch, lactose, glucose, sodium alginate, calcium gluconate and magnesium stearate was tested for possible interference in the assay by placebo blank. It was confirmed that the change in the titrant value and the absorbance with respect to the water blank was caused only by the analyte. A separate test was performed by applying the proposed methods to the determination of CBZ in a synthetic mixture and the percent recoveries of CBZ were 101.3 ± 2.13, 98.65 ± 1.96 and 97.24 ± 2.02 (n = 5) by titrimetric, spectrophotometric method A and method B, respectively, suggesting that no significant interference by the excipients in the assay of CBZ under the described optimum conditions.

3.3.4. Application to assay of pharmaceutical formulations and spiked urine sample

The proposed methods were successfully applied to the determination of CBZ in formulations (tablet and syrup) (Table 4). The results obtained were statistically compared with those of the official method (British Pharmacopoeia, 1973) by applying the Student's t-test for accuracy and F-test for precision. The official method consisted of measurement of the absorbance of the alcoholic extract of the tablets at 285 nm. From Table 4, it is clear that the calculated t-value and F-value at 95% confidence level did not exceed the tabulated values of 2.78 and 6.39, respectively, for four degrees of freedom. The results indicated that there is no difference between the proposed methods and the official method with respect to accuracy and precision. The proposed spectrophotometric methods were also applied to the determination of CBZ in spiked human urine sample and the results are presented in Table 5. Accuracy and validity of the proposed methods were further ascertained performing recovery experiments *via* the standard addition procedure. When the tablet powder or syrup (pre-analyzed) spiked with known amounts of pure CBZ at three different concentration levels (50%, 100% and 150% of the quantity present in the tablet powder or syrup) was analyzed by the proposed methods, the recoveries of pure drug added were quantitative (Table 6).

Table 6 Results of recovery experiments using the standard addition method.

Formulation studied	Titrimetric method				Spectrophotometric (method A)				Spectrophotometric (method B)			
	CBZ in formulation (mg)	Pure CBZ added (mg)	Total found (mg)	Pure CBZ recovered[a] (percent ± SD)	CBZ in formulation (μg ml^{-1})	Pure CBZ added (μg ml^{-1})	Total found (μg ml^{-1})	Pure CBZ recovered[a] (percent ± SD)	CBZ in formulation (μg ml^{-1})	Pure CBZ added (μg ml^{-1})	Total found (μg ml^{-1})	Pure CBZ recovered[a] (percent ± SD)
Zeptol tablet (100 mg)	1.95	1.0	2.98	103.0 ± 1.23	0.50	0.25	0.74	96.00 ± 2.06	1.98	1.0	2.97	99.00 ± 1.26
	1.95	2.0	3.86	95.50 ± 1.76	0.50	0.50	1.01	102.0 ± 1.06	1.98	2.0	3.89	95.50 ± 1.88
	1.95	3.0	4.84	96.33 ± 1.17	0.50	0.75	1.23	97.33 ± 2.74	1.98	3.0	4.90	97.33 ± 0.97
Tegrital syrup (100 mg/5 ml)	1.91	1.0	2.90	99.00 ± 2.47	0.48	0.25	0.74	104.0 ± 2.31	1.95	1.0	2.92	97.00 ± 1.99
	1.91	2.0	3.86	97.50 ± 1.98	0.48	0.50	0.96	96.00 ± 1.77	1.95	2.0	3.86	95.50 ± 2.15
	1.91	3.0	4.90	99.67 ± 1.27	0.48	0.75	1.22	98.67 ± 1.43	1.95	3.0	4.84	96.33 ± 2.69

[a] Mean value of three determinations.

4. Conclusion

This is the first report on the assay of carbamazepine by titrimetry and the third by visible spectrophotometry. In particular, the titrimetry is much simpler in technique, more rapid than all the methods reported so far for carbamazepine and it is applicable over a micro range (1.00–7.50 mg), yet provides very accurate and precise results. The proposed spectrophotometric methods are highly sensitive than many reported methods such as HPLC (Demirkaya and Kadioglu, 2005, 2008) [0.25–25 μg ml^{-1}], HPLC (Yuan et al., 2003) [5.0–25 μg ml^{-1}], LC (Predrag et al., 2009) [100–500 μg ml^{-1}], GC (Kadioglu and Demirkaya, 2007) [2.0–30 μg ml^{-1}], UV-spectrophotometry (Tatar Ulu, 2006) [4.0–10 μg ml^{-1}], UV-spectrophotometry (Demirkaya and Kadioglu, 2008) [1.25–25.0 μg ml^{-1}], visible spectrophotometry (Rao and Murty, 1982) [10.0–100 μg ml^{-1}] and visible spectrophotometry (Agrawal et al., 1989) [4.0–80 μg ml^{-1}]. Moreover, the proposed methods can be performed at milder experimental conditions without heating or extraction step. The proposed methods rely on the use of simple, cheap and readily available chemicals and techniques but provide a sensitivity comparable to that achieved by sophisticated and expensive technique like HPLC. Thus, they can be used as alternatives for rapid and routine determination of bulk sample and formulations.

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