

**CONTENT VARIATIONS OF CARBAMAZEPINE TABLETS IN IRAQI COMMUNITY PHARMACIES:
APPLICATION OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY ASSAY METHOD**

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Received: 22 September 2017, Revised and Accepted: 30 October 2018

ABSTRACT

Objective: There is paucity in the literature and documentation regarding the post-marketing product quality in Iraq and Middle East countries in general. Therefore, a simple reverse phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the determination of carbamazepine (CMZ) content in post-marketed tablet dosage form.

Methods: The RP-HPLC analysis was run at a flow rate of 1 ml/min with a mixture of methanol: water (70:30) mobile phase using a Thermo Synchronise C18 column at a UV detection wavelength of 230 nm. The method was validated for selectivity, linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), solution stability, and robustness.

Results: The calibration curve was linear over a concentration range of 1–20 µg/ml ($r^2=0.9999$) with LOD and LOQ of 0.01 and 0.04 µg/ml, respectively. The intraday and interday precision and accuracy were between 0.04–1.67% and 0.04–0.78%, respectively. The results of analysis of the four brands of CMZ tablets were passed the European Pharmacopoeia specification but with a high standard deviation of mean drug content of some brands.

Conclusion: This study shows that the quality of post-marketed drugs and/or the storage conditions in developing countries must be carefully assessed for a better therapeutic response.

Keywords: Carbamazepine, Drug content, High-performance liquid chromatography, Tablet quality.

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INTRODUCTION

Substandard drug is a genuine product but has incorrect amount of active pharmaceutical ingredient [1-3]. This may be due to poor manufacturing quality or excessive decomposition of active ingredient [4], while counterfeit drugs have defined by the World Health Organization as those that are "deliberately and fraudulently mislabeled with respect to identity and/or source" [5]. Poor regulation of pharmaceutical companies, coordinated criminal organization, and system corruption was linked with the counterfeit drug trading [6].

In Iraq, although the rigorous order for licensing of import and/or manufacture of drugs, the complex transactions involving many intermediaries, lack of regulation in many border sites and high demand make the market for substandard drugs more lucrative one. This study is the first one in this field in Iraq and Middle East countries. It is a pilot study to assess carbamazepine (CMZ) tablet content in Iraqi markets. CMZ is an antiepileptic drug (AED), and substandard formulations of CMZ will have serious effects. The main ones are being treatment failures, prolonged illnesses, and unexpected side effects. Therefore, the aims of this study are to develop a simple and sensitive reverse phase-high performance liquid chromatography (RP-HPLC) method to analyze CMZ and to use the developed method in the quality control analysis.

METHODS**Purchasing samples**

Four marketed brands of CMZ tablets were commonly available in the community pharmacies. 10 strips for each brand were purchased (1 strip from each pharmacy) and named as Batch 1, 2, 3, and 4 (B1, B2, B3, and B4). The samples were collected by local collaborators who had no idea about the purpose of the purchases.

Materials and chromatographic conditions

The HPLC system used in this study was Shimadzu with a UV/Vis detector (SPD-20A; Shimadzu, Japan). The analyte separation was accomplished at 40°C (CTO-10AS VP, Shimadzu column oven) using a Synchronise C18 analytical column, 150 × 4.6 mm ID, 5 µm (Thermo, USA) with a mobile phase mixture of methanol:water (70:30). The mobile phase was filtered under vacuum through a 0.45 µm nylon filter (Whatman, UK) and degassed before use. A flow rate and wavelength were set at 1.0 ml/min and 230 nm, respectively. The injection volume was 20 µL. CMZ was obtained from Tokyo Chemical Industry Co., LTD (Tokyo, Japan) and methanol HPLC grade was obtained from J. T. Baker Analyzed (Shanghai, China).

Solutions for the validation study

A stock solution of 1 mg/ml of CMZ in methanol was prepared. The working solutions containing 1 to 20 µg/ml of CMZ were prepared, and 20 µL aliquots were injected 6 times and eluted under the reported chromatographic conditions. For regression equation calculation, the average peak area versus the concentration of CMZ in µg/ml was plotted.

Preparation of the sample solution

CMZ tablet was crushed to a homogeneous powder then dissolved in 50 ml of methanol in 100 ml volumetric flask with the help of sonicator. Thereafter, the volume was made up to mark with methanol and filtered through a 0.45 µm nylon membrane filter (Whatman, UK) under vacuum. The filtrate was further diluted to obtain sample solutions of concentrations within the calibration curve range.

METHOD VALIDATION**Selectivity**

The selectivity of the developed method was determined by checking the interference between the spectral purity obtained from working

standard solution and sample solution containing excipients that present in the pharmaceutical products.

Linearity

The linearity of the method was evaluated using six calibration curves in the concentration range of 1–20 µg/ml. The linear regression analysis method was used to construct the calibration curves plot (peak area of the analyte against the corresponding concentration).

Precision

Repeatability and reproducibility were evaluated for method precision. Quality control samples of 1, 5, and 20 µg/ml (low, medium, and high concentrations, respectively) were analyzed to evaluate intra- and inter-day precision. Six replicates of three concentration levels on the same day and 3 different days were analyzed for intra- and inter-day precision, respectively.

Accuracy

A standard addition method was used to assess the accuracy of the method. The addition method was performed by adding a known amount of standard drug to 80%, 100%, and 120% of the target test concentrations of the pre-analyzed tablet formulation. Three determinations were performed for each level.

Limit of detection (LOD) and limit of quantification (LOQ)

The standard deviation and slope of the response were used to determine the sensitivity of the method as described in ICH guidelines Q2 (R1) [7]. The LOD and LOQ were calculated according to the following equations:

$$\text{LOD} = 3.3 \sigma/S; \text{LOQ} = 10 \sigma/S$$

Where σ = the standard deviation of the response; S = the slope of the calibration curve.

Solution stability

Reference solutions with low, medium, and high-quality control samples were stored in the refrigerator for 14 days. Then, the samples were re-analyzed by employing freshly prepared standard solutions.

Method robustness

The method robustness was assessed as a function of altering methanol and water mobile phase ratio over a range of $\pm 5\%$. The concentration of solution analyzed was 20 µg/ml (n=6).

RESULTS AND DISCUSSION

Method optimization

The mobile phase (methanol: water) was chosen due to its availability, low cost, and gave good peak resolution. In addition, using water can increase the lifespan of the column if compared to buffer salt [8].

Method validation

As described in ICH guideline Q2 (R1) any newly developed method must be validated before its use for the intended [7]. For this purpose, this method was validated for its selectivity, linearity, precision, accuracy, LOD, LOQ, solution stability, and robustness.

Selectivity

The chromatographic condition revealed to be selective for CMZ. Fig. 1a and b show a typical separation of CMZ (20 µg/ml) with no interfering peaks from mobile phase, respectively. Moreover, there were insignificant interfering peaks from sample solution (containing excipients) with CMZ at a retention time of 2.5 min (Fig. 1c) which indicate the selectivity of the developed method. Therefore, the results

showed that the newly developed method was appropriate for quality control analysis.

Linearity

The linear regression equation with a correlation coefficient of 0.9999 was $y = 100635 (\pm 359) \times + 63270 (\pm 1006)$. This result revealed an excellent correlation between the peak area and concentration of the analyte. The linearity results are presented in Table 1.

Precision

The relative standard deviation percent (% RSD) for intraday and interday precision was ranged from 0.04 to 1.67. The RSD% of the peak area of six replicates was found to be <2% and within the acceptable limits [9]. The results of precision are shown in Table 2.

Accuracy

The results of the recovery study are shown in Table 3. All the results were within the acceptable limits which indicate the accuracy of the validated method to detect CMZ in tablet formulation [9].

LOD and LOQ

The LOD and LOQ were found to be 0.01 µg/ml and 0.04 µg/ml, respectively. The results of LOD and LOQ were indicating a high sensitivity of the method.

Solution stability

The results of the stability test are shown in Table 2. The results revealed that the drug was stable in the above-mentioned condition which was sufficient to complete the whole analytical process.

Table 1: Summary of calibration curve results for CMZ, n=6

Theoretical CMZ concentration µg/ml	RSD (%)*	RE (%)**
20	0.05	0.06
10	0.06	0.4
5	0.1	0.9
2	0.8	1.2
1	1.4	1.89

*RSD%, relative standard deviation percent, **RE%, relative error percent, CMZ: Carbamazepine

Table 2: Experimental values of mean concentration of CMZ, RSD (%) presented for validation parameters of CMZ

Parameters	Theoretical CMZ concentration µg/ml	% RSD
Intraday	20	0.04
	5	0.2
	1	1.5
Interday	20	0.09
	5	1.1
	1	1.67
Short-term stability	20	0.50
	5	1.50
	1	1.04

CMZ: Carbamazepine, RSD: Relative standard deviation

Table 3: Recovery data for CMZ of four different batches

% of target concentration	Mean % recovery	RSD %
80	96.44	0.78
100	101.11	0.04
120	98.76	0.10

100% of the target concentration is equivalent to 10 µg/ml of three replicates. CMZ: carbamazepine, RSD: Relative standard deviation

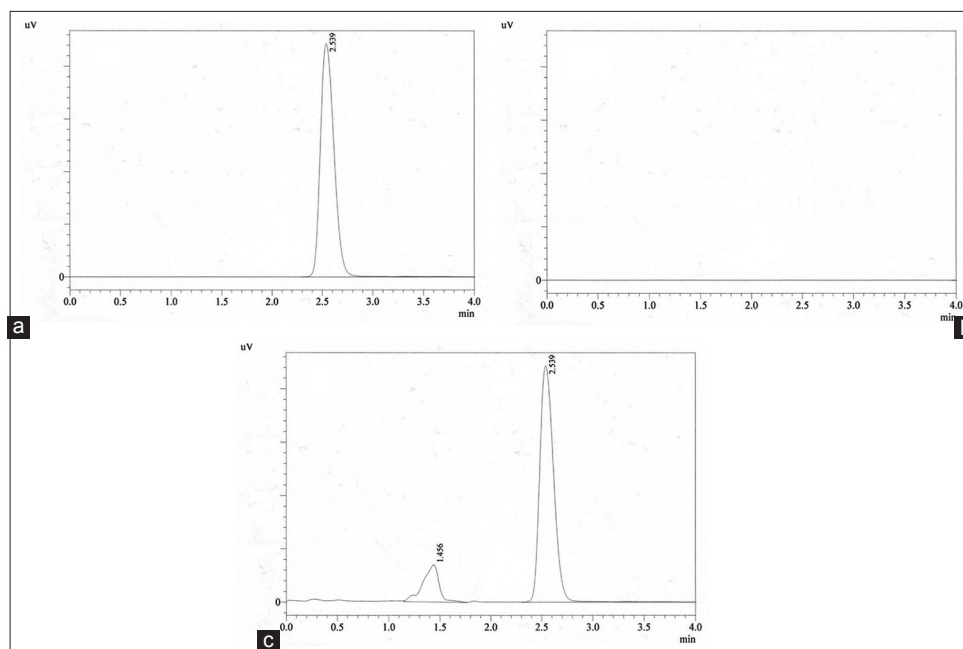


Fig. 1: Characteristic high-performance liquid chromatography-ultraviolet chromatograms of carbamazepine (CMZ). (a) Spiked placebo, (b) mobile phase, and (c) sample solution; the retention time of CMZ is 2.5 min

Robustness

There were unpredictable changes in the chromatograms demonstrated with % RSD range (0.6–1.1%). The low values of the %RSD indicated the robustness of the method.

Method application

Four marketed CMZ tablets were analyzed for drug content (B1, B2, B3, and B4). According to European Pharmacopoeia [10], the criteria for drug content states that CMZ tablets must contain not <85% and not more than 115% of CMZ stated on the label. The results of analysis of the four brands of CMZ tablets are represented in Table 4.

From the results, all batches passed the EP specification. However, the results showed higher standard deviation for Carbasam® and Tegral® compared to Tegretol® and Taver® tablets. This is due high variation in drug content in B2 and B3 samples (data not shown). Many reasons could affect this result. Inadequate production process validation and quality control during the manufacturing process may be the main reason.

This result is consistent with the previous report of the quality of antimalarial drugs [11]. In addition, Carbasam® tablets were stored in a box containing more than 100 strips and soiled to pharmacies as individual strips which result in them to be more exposed to heat, light, and humidity. Moreover, the type of the packing strip is another factor needed to be address [12,13]. In addition to the above, in Iraq, power outage (more than 15 h in hot summer) is also play a role in these results. However, further studies were needed to assess these factors.

Finally, this study shows that the quality of post-marketed drugs and/or the storage conditions in developing countries must be carefully assessed for a better therapeutic response. In addition, the AED quality is important because seizure is a chronic disease and concerns a lot of individuals in developing countries. Moreover, development of a simple method for detection any therapeutic agent is important for industrial application so can improve production processes [14,15].

CONCLUSION

A simple and sensitive RP-HPLC method was developed for the determination of CMZ in tablet dosage forms. All validation parameters tested results were satisfactory. The method is cost-effective due to

Table 4: Assay results of carbamazepine tablets

Batch	Brand name	Average content (%), n=100
B1	Tegretol®	96.33±2.66
B2	Carbasam®	95.43±9.32
B3	Tegral®	93.86±6.11
B4	Taver®	95.33±3.63

short retention time which allowed a large number of samples to be analyzed within a short period of time. The developed method was successfully applied for drug content analysis of commercially available CMZ tablets. All batches passed the drug content criteria but with a high variation which may cause inadequate control of seizures and tolerance developments to AED which may be attributed to the quality aspect of the drug.

DECLARATION

The author confirms that the manuscript has not been submitted to any other Journal for publication

CONFLICTS OF INTERESTS

The author has none to declare.

AUTHOR'S CONTRIBUTIONS

Sahib MN is responsible for study design, data acquisition, and manuscript preparation.

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