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RP-HPLC method for the simultaneous determination of carbamazepine and nilotinib: Application solubility studies

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Abstract A simple and precise high performance liquid chromatography method for concurrently determination of nilotinib (NTB) and carbamazepine (CBZ) in their pharmaceutical formulations was developed and validated. β-estradiol was used as an internal standard. The separation was carried out on Phenomenex Luna C_{18} RP-HPLC column using an isocratic elution at 1 mL/min flow rate with water- acetonitrile (30:70 v/v) mobile phase. Quantification was performed with a UV photodiode array detector at 286 nm. Solubility of NTB and CBZ was determined individually and their enhancing/reducing effect on solubility of each other in simulated biological fluids at various pHs was investigated. The calibration curves for each drug were linear in the range of 0.0125-5 µg/mL. The developed method was applied to pharmaceutical formulation successfully with no interfering peaks. The percentage recovery was 98.13% for CBZ and 96.25 % for NTB. It was observed that solubility of CBZ was increased in all *in vitro* interaction medium in the presence of NTB while solubility of NTB was increased mostly in pH 6.8 and slightly in pH 1.2.

Keywords Nilotinib; carbamazepine; HPLC; determination; solubility.

Introduction

Nilotinib (NTB) (Fig. 1) is a tyrosine kinase inhibitor used orally to treat chronic myeloid leukemia. NTB prevents intracellular signal transduction protein the downstream phosphorylation**.** NTB is well absorbed and reached the maximum peak plasma concentration in 3 h after oral administration. Bioavailability of NTB is about 30%. The binding of NTB to plasma protein is about 99%[1]. Various instrumental techniques have been reported for the determination of NTB alone and in combine with other anti-chronic myeloid leukemia agents in biological matrixes by high performance liquid chromatography- ultraviolet (HPLC-UV) [2-5], Ultra performance liquid chromatography (UPLC) [6], HPLC-mass spectrometry (MS) [7], Ultra-performance liquid chromatography-tandem mass (UPLC -MS/MS) [8] and HPLC-MS/MS [9-10]. In the literature, there are two RPLC-UV method for determination of NTB in its capsules [11-12].

Carbamazepine (CBZ) (Fig. 1) is an anticonvulsant drug used to treat simple and complex partial seizures, tonic-clonic seizures. CBZ is also effective in the treatment of trigeminal neuralgia pain, certain psychiatric disorders such as manic-depressive illness. The mechanism of anticonvulsant effect of carbamazepine is not fully understood. The basic mechanism of pain relief of carbamazepine is believed to be associated with blocking of synaptic transmission in the trigeminal nucleus [13]. Maximum peak plasma concentration occurs in 3 h after oral administration. Bioavailability and protein binding rate are about 85% and 70%, respectively. Toxicity symptoms of CBZ generally occur when the plasma concentrations excess 10-12 mg/L [13]. Various instrumental techniques have been

published for the determination of CBZ in biological matrices and pharmaceutical formulations, including HPLC-UV [14-17] voltammetry [18], micellar electrokinetic chromatography [19], micellar ion chromatography [20] and gas chromatographic methods [14, 21]. However, no method for simultaneous determination of NTB and CBZ has been published so far.

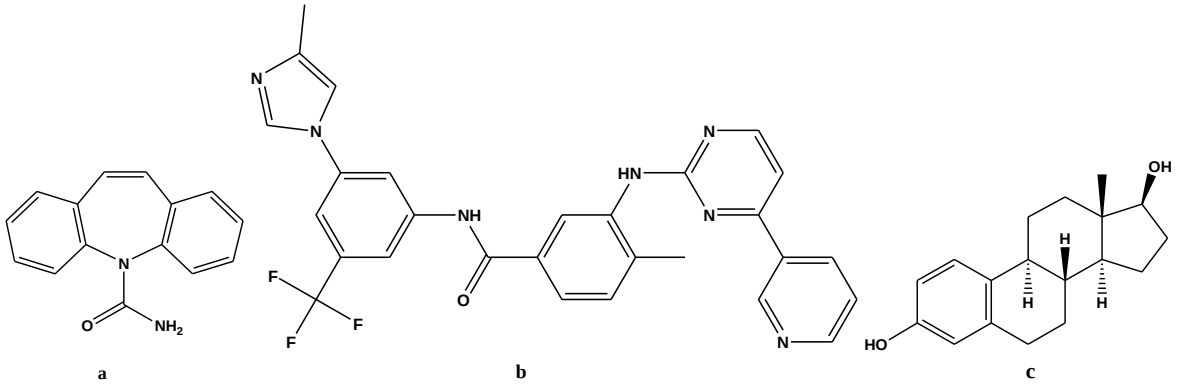

Figure 1: Chemical structure of CBZ (a), NTB (b) and β-estradiol (c).

Drug-drug interactions can occur in the cancer treatment since multiple drugs are used to treat or prevent the complications related to disease besides the main treatment. Among these complications, psychological disorders and neuropathic pain are quite common [22-24]. CBZ widely used as anticonvulsant drug because of its favorable therapeutic profile [13].

NTB and CBZ have a narrow therapeutic range and therefore both need to be monitored for toxicity and adjusting the treatment of dose. NTB is metabolized by CYP3A4 with oxidation and hydroxylation [24-25]. CBZ has strong CYP3A4 inducer feature. CBZ is mainly metabolized by CYP3A4 to an active metabolite having same activity [26]. Concurrent use of NTB with CBZ may result in increased plasma concentration of CBZ and reduced plasma level of the NTB by accelerating its metabolism. Thereby, the dose of NTB may need to be increased to achieve the same effect. This enhances the toxicity of drug [24].

The solubility and dissolution of drugs in gastrointestinal fluids is a key indicator for oral bioavailability. To exhibit pharmacological activity, the molecule must be soluble in physiological of fluids to be absorbed. Moreover, solubility profile of drugs is needed to determine potential problems for drug precipitation *in vivo* [27].

Both CBZ and NTB are weak bases and belong to the class II and IV, respectively of the biopharmaceutical classification system.CBZ is sparingly soluble in aqueous system and faster converted to the dehydrate form. Although solubility of CBZ is not pH dependent since it is non-ionizing compound, its solubility may change in different solution with other compounds. This showed that absorption of CBZ is irregular [28-30]. Solubility of NTB is increases with decreasing pH. Thus, significant drug interactions in gastrointestinal tract may occur when NTB and CBZ are administered at the same time. This interaction can cause *alterations* of their pharmacokinetic properties that are involved absorption, distribution, and metabolism [30-31]. In literature, there are some *in vitro* dissolution studies of CBZ in pharmaceutical dosage form and together with some compounds [28, 30, 32-33]. Also, some *in vivo* interaction studies have been reported on the interaction of pharmaceutical dosage form and food with CBZ [13, 26, 29] and with NTB [25, 34-35]. However, there is no *in vitro* study based on the pH dependent solubility for both drugs in the mixture.

For these reasons, the purpose of this research is to develop a sensitive, easy, cost- effective and rapid isocratic RP-HPLC with photodiode array (DAD) detection method for the simultaneous determination of NTB and CBZ in a bulk material and their pharmaceutical formulations for the first time. Moreover, the developed method was applied to solubility studies to evaluate how both drugs affect solubility of each other using simulated biological fluids at different pHs, including empty and full gastric juice (pH 1.2 and 4), intestine (pH 6.8 and 9.0) and blood pH (7.4) at 37 ^{o}C for 24 h. These solubility experimental results may contribute to understand amount of interaction between CBZ and NTB and *in vivo* studies. The developed method was validated according to ICH guideline [36].

Materials and Methods

Chemicals and Materials

CBZ and NTB were kindly supplied by Novartis pharmaceuticals (Istanbul, Turkey). 17β-Estradiol (EST) (Fig. 1) was obtained from Sigma Aldrich. All solvents and chemicals with HPLC grade were purchased from Merck. Tegretol tablets containing CBZ were purchased from a local pharmacy. A WTW pH 526 digital pH meter (Mettler Toledo, Switzerland) was used in the pH measurements. A shaking water bath (WiseBath, Feedback Control Digital Timer Function, Sweden) was used for solubility studies. Water used in all analyzes was obtained using an aqua MAXTM-ultra water purification system (Young-Lin Instrument, South Korea).

Instrumentation and Chromatographic Conditions

Analysis was carried out on a Thermo Separation system (San Jose, CA) with P 4000 pump, SN 4000 system controller, UV 6000 LP detector, 20 µL sample loop and ChromQuest 5.0 software. The photodiode array detector set at 286 nm wavelength.

Separations were performed on a C_{18}-column (250 mm × 4.6 mm i.d. 5µm, Phenomenex, Luna). A guard column with the same packaging material was used. Water and acetonitrile (30:70 *v/v*) was used as mobile phase in isocratic mode with 1 mL/min flow rate at room temperature. The mobile phase was filtered with a Millipore vacuum filtration, fitted with a HV 0.45 µm filter.

Solutions

Stock solutions of NTB (expressed as base compound) and CBZ at 0.5 mg/mL concentrations in methanol were prepared separately. The preparation of working standard solutions of both drugs at 0.025 mg/mL was made separately by appropriate dilutions from stock solution in water-acetonitrile (30:70, *v/v*).

A stock solution of 17β-estradiol as internal standard (IS) at 1.0 mg / mL concentration was prepared in methanol and the working solution at 0.01 mg/mL was prepared by appropriate dilution from stock solution in the mobile phase. The stock solutions remained stable for more than two weeks in the refrigerator.

Buffers

pH 1.2 buffer (simulated gastric fluid without enzymes):The buffer was made by dissolving 3.73 g potassium chloride inappropriate amount of water. After adding 7.0 mL of concentrated hydrochloric acid, the solution pH was adjusted with 0.1 M NaOH or 0.1 M HCl and completed to 1.0 L volume with water.

Phosphate buffers (pH 4.0, 6.8, 7.4 and 9.0): For preparation of buffers, 6.8 g potassium dihydrogen phosphate and 0.9 g sodium hydroxide were dissolved in 1.0 L round bottom flask with a sufficient quantity of water. After adjusting the pH value, the volume was completed with water [37].

Procedure for Calibration Curves

Standard solutions of CBZ and NTB at the concentrations of 0.0125, 0.025, 0.05, 0.1, 0.25, 0.5, 1.0, 2.0 and 5.0 µg/mL in the mixture were prepared by appropriate dilution of their stock solutions in mobile phase. 250 µL β-Estradiol (10 µg /mL) as an internal standard (IS) was added to each analysis samples prior to completion to volume. Five independent determinations were carried out for each concentration. Calibration curves were obtained by plotting the peak area of CBZ or NTB ratio to IS versus their concentrations and the least squares regression analysis was obtained.

Determination of CBZ and NTB in Pharmaceutical Formulations

Capsules containing 200 mg of NTB were prepared in the laboratory of the Department of Pharmaceutical Technology at our faculty because it was unable to buy. The capsule contains the following ingredients: microcrystalline cellulose, lactose monohydrate, magnesium stearate, *crospovidone*, *poloxamer 188*, colloidal silicon *dioxide* and *Polyplasdone®XL-10*.

Five capsules of NTB and 8 tablets of CBZ (Tegretol®, 200 mg/tablet) were weighed and powdered separately. Amount of the powders equivalent to 200 mg of drugs were transferred to a 50 mL volumetric flask with 30 mL of methanol separately and then shaken in an ultrasonic bath at room temperature for 1 h. The volumes were completed to 50 mL with methanol and filtered. The solution of capsules and tablets were analyzed as in section

"Procedure for calibration curves" after addition of β-estradiol as IS and appropriate dilutions with mobile phase. Content of the capsules and tablets were found using related regression equations of the calibration curves.

Solubility Studies of CBZ and NTB Individually and Together

Quantitities of CBZ and NTB were individually and in their binary mixtures were prepared into a series of vials in the pH of 1.2, 4.5, 6.8, 7.4 and pH 9.0 medium (totally 15 samples). Drugs were added into the vials containing mediums which were greater than the quantity expected to dissolve. 6 mg of each substance were transferred into vials containing 10 mL of buffer (concentration of each drug was 600 µg/mL). The samples prepared in vials were allowed in a shaking water bath at 37 °C with constant horizontal agitation at 200 rpm for 24 hours. After 24 h incubation, sample solutions in each vial were filtered through a 0.25 µm membrane filter and dilution in mobile phase was made to obtain for 3 µg/mL solutions. Then, each sample contents were analyzed in triplicate by proposed method. The concentration of samples was calculated by related regression equations of calibration curves for standard drug solutions. Solubility interaction percentage of CBZ and NTB was calculated by comparing the recovery of results that obtained by combination of both drugs samples with the drug samples alone in the same pH medium.

Results

Optimization of Separation Conditions

The adequate mobile phase and relevant chromatographic conditions were determined taking into account peak resolution, retention time, and symmetry of peaks. Water-acetonitrile and water-methanol mixtures in various ratios (30:70, 35:65 and 40:60 v/v) were tested as mobile phases. The best mobile phase was found to be 30:70 water-acetonitrile as eluent in isocratic mode, which proved excellent separation of drugs within approx. 3.5, 4.36 and 5.5 min for CBZ and IS and NTB, respectively. RSD % of the retention times was about % 0.13, 0.18 and 0.49 for CBZ, IS and NTB, respectively (for 10 independent analyses). Besides, the system suitability was examined by tailing factor, number of theoretical plates, capacity and separation factor and resolution of both analytes together with IS and found to be quite well suitability of system (Table 1).

Table 1: System performance parameters of NTB, CBZ and IS

Parameter	NTB	CBZ	IS
Retention time, tr	5.50	3.50	4.36
Capacity factor, k'	1.50	0.59	0.98
Resolution, Rs	4.40	6.53	4.91
Tailing factor, T	1.26	1.14	1.15
Therotical plates, N	4596	8114	7971
Seperation factor	1.53	20,49	1.66
Plates / meter	18383.56	32454.24	31885.84

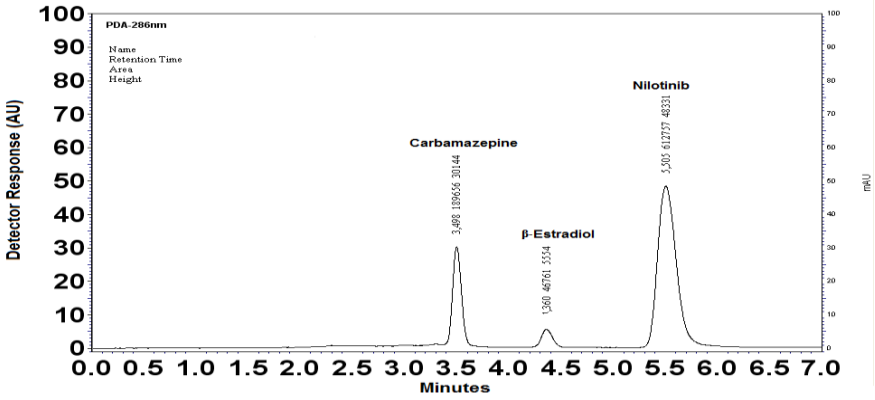

Figure 2: A typical chromatogram of CBZ, NTB with IS in the assay conditions ([CBZ]=[NTB]= 2.0 µg/mL and [IS]= 2.5 µg/mL).

A Phenomenex C_{18} and Venusil XBP C_{18} (Agela) columns were tried to obtain the best separation and Phenomenex C_{18} column was found to give well performance. For wavelength selection, the mixture of drugs in different mobile phase systems was scanned by a UV-Vis spectrophotometer and also by the HPLC-DAD system. For all analytes, optimum wavelength and flow rate at room temperature were 286 nm and 1 mL/min, respectively. The various drug substances were tested as an IS and β-estradiol was selected due to its appropriate retention time and symmetry. Typical chromatogram of CBZ and NTB with IS shown in Figure 2.

Method Validation

The developed method was validated with respect to linearity, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy and stability according to ICH guideline [36].

Linearity and Sensitivity

The parameters for calibration curves under the selected conditions are summarized in Table 2. Linear range for both drugs was 0.0125 - 5 µg/mL. Correlation coefficients (r^2) for drugs were 0.9997, showing good linearity (Table 2).

Limit of detection (LOD) and quantification (LOQ) of CBZ and NTB for developed method were calculated by the following equation: LOD=3.3 S_a/b and LOQ=10 S_a/b, where S_a is the standard deviation of the intercept and b is the slope of calibration curve [36]. The values of LOD and LOQ were 0.004 and 0.011 µg/mL for CBZ, 0.004 and 0.012 µg/mL for NTB, respectively (Table 2).

Table 2: Statistical parameters for analysis of CBZ and NTB

Parameters	CBZ	NTB
Linearity range (µg/mL)	0.0125-5.0	0.0125-5.0
Regression equation[a]		
Slope	2.0992	6.8688
Intercept	0.0739	0.0458
Correlation coefficient (r^2)	0.9997	0.9997
SD of a	0.005	0.007
SD of b	0.002	0.008
LOD (µg/mL)	0.004	0.004
LOQ (µg/mL)	0.011	0.012

[a] $y = aC + b$ (C is the concentration of drug in µg/mL, y is peak area, a is slope and b is intercept).

Table 3: Intra-day and inter-day precision and accuracy of CBZ and NTB.

	Added concentration (µg/ mL)	Found concentration (µg/mL) mean ± SD	Precision (RSD %)	Accuracy (%)
CBZ	Intra-day			
	0.25	0.2485 ± 0.0010	0.40	99.4
	0.5	0.5211 ± 0.0149	2.85	104.22
	2	2.0394 ± 0.0278	1.36	101.97
	Inter-day			
	0.25	0.2404 ± 0.0067	2.79	96.16
	0.5	0.5241 ± 0.0170	3.24	104.82
	2	2.0434 ± 0.0306	1.50	102.17
NTB	Intra-day			
	0.25	0.2609 ± 0.0077	2.95	104.36
	0.5	0.4782 ± 0.0154	3.22	95.64
	2	2.0406 ± 0.0287	1.41	102.03
	Inter-day			
	0.25	0.2405 ± 0.0067	2.79	96.2
	0.5	0.4689 ± 0.0219	4.67	93.78
	2	2.0434 ± 0.0306	1.50	102.17

Accuracy and Precision

To assess precision and accuracy, samples at 0.25, 0.5 and 2 μg/mL concentrations for both drugs were analyzed within one day (intra-day) and in different days (inter-day). Five independent analyses were performed in selected conditions to identify intra-day and inter-day precision. The percent relative standard deviation (RSD %) values for intra-day and inter-day precision were in the range of 0.40-2.85% and 1.50-3.24% for CBZ, and 1.41-3.22 and 1.50-4.67% for NTB, respectively (Table 3).

Stability

To examine stability, CBZ and NTB solution in the mixture was analyzed after stored at +4 ^{o}C in refrigerate for one month and in the dark for about one week at room temperature. The assay was repeated three times. The results of analysis were compared with results of freshly prepared drug solutions and the CBZ was found to be stable for one month, while NTB under these conditions found stable for 2 weeks.

Discussion

Comparison of the Developed Method with Literature Methods

HPLC is a powerful analytical technique most commonly used in science and in a variety of industry to the separation and determination of water-soluble molecules. There are many reasons for the application of the HPLC method: It plays a major role in drug preparation, determination of drug substances in various matrices, pharmacokinetic studies, and stability and in drug interaction studies because of its rapidity, selectivity, sensitivity and use of automation. HPLC technique, especially RP-HPLC coupled to UV detection is widely used for separation and determination of active ingredients concentration without interference from other drugs and impurities. RP-HPLC is common used in the simultaneous determination of drugs and their metabolites in complex samples in a single analysis with the high resolution, reproducibility and specificity in short time [38-39].

The RP-HPLC method has several advantages over other reported methods. Recommended isocratic elution with water-acetonitrile method was more simple, robust and gave minimal baseline disturbances compared to reported gradient elution methods containing acid additives [6, 40-41]. The method provides a wide linearity range for both drugs. The sensitivity and retention time of the proposed method for CBZ compared most favorably to reported HPLC techniques [15, 16, 40-47].

Retention time of NTB was found shorter than the published methods [2, 4-5]. Besides, the sensitivity of NTB was higher than those of reported by other studies [4, 8, 48-49].

Applications to Pharmaceutical Formulations

The developed method was applied to determination of CBZ and NTB in their tablets and capsules, respectively. Each tablet/capsule contains 200 mg pure compound. The mean recovery values were 98.13% (RSD % = 1.33) and 96.25 % (RSD % = 2.72) for CBZ and NTB, respectively (Table 4).

Table 4: Analysis of CBZ and NTB in pharmaceutical formulations by developed method (200 mg per tablet or capsules for both drugs, n=6).

Statistical Values	CBZ	NTB
Mean (mg) ± SD	196.25 ± 2.61	192.5 ± 5.23
Recovery (%)	98.13	96.25
RSD (%)	1.33	2.72

Solubility Studies

In vitro interaction studies provide benefits regarding to preliminary understanding release, solubility, dissolution, and absorption profiles of drugs to guide *in vivo* study and reduce a large number of studies on humans [50].

The aim of this solubility study was to determine the potential enhancement/decrease solubility effect of both drugs on each other in different pH medium by proposed method.

In this study, NTB and CBZ were subjected to five different simulating body fluids of pH 1,2, 4, 6.8, 7,4 and 9 for *in vitro* evaluation of their solubility individually and in the binary mixture. The sample contents were quantitated by proposed method. Effects of pH on the solubility of NTB and CBZ in the presence of each other were evaluated. In order to obtain apparent solubility and avoid the influence of excipients interaction, raw drugs were used. The

interaction study was carried out for 24 h since NTB have poor solubility in aqueous solution. The results of % solubility of CBZ and NTB individually and in their combination at different pHs was shown in (Table 5).

Table 5: Results of solubility studies of CBZ and NTB at various pHs.

Drug	**Drugs alone**		**Drugs together**	
	CBZ	NTB	CBZ	NTB
	pH = 1.2			
% Solubility	79.23	84.79	133.48	92.31
%RSD	2.44	0.79	0.93	1.51
Interaction amount % (±)			68.47	8.87
	pH = 4			
% Solubility	84.41	0.15	99.28	0.14
%RSD	0.30	0.32	2.13	3.00
Interaction amount % (±)			17.62	6.66
	pH = 6.8			
% Solubility	32.43	0.51	77.40	1.22
%RSD	3.12	2.03	0.91	2.35
Interaction amount % (±)			138.67	139.22
	pH = 7.4			
% Solubility	24.65	2.01	69.64	0.10
%RSD	0.78	2.24	1.26	1.89
Interaction amount % (±)			182.52	95.02
	pH = 9			
% Solubility	18.20	1.00	34.30	0.73
%RSD	1.83	3.00	2.26	1.24
Interaction amount % (±)			86.46	27.00

In presence of NTB, %solubility of CBZ increased in all pH medium, which may lead over dosage of CBZ and causing side effects. In combination, the availability of CBZ was highest in pH of 1.2 medium with 133.48% and lowest in pH of 9 with 34.30%. Further, the percent solubility of CBZ in pH of 7.4, 6.8, 9, 1.2 and pH 4 showed an increase with the percentage of 182.52, 138.67, 86.46 68.47 and of 17.62%, respectively compared to those when it alone (Table 5).

In presence of CBZ, solubility of NTB was highest at pH 1.2 with the percentage of 92.31%, compared with its result (84.79%) treated alone. Also, recovery percentage of NTB at pH 6.8 increased from 0.51% to 1.22% by showing an increase of 139.22%. However, the solubility of NTB decreased at pH of 7.4, 9 and pH 4 with the percentage of 95.02, 27.00 and 6.66 %, respectively (Table 5). The results of these tests indicated that both drugs affect the solubility of each other. However, *in vivo* studies may necessary for a comprehensive determination the interaction rate of NTB with CBZ on gastrointestinal absorption since their solubilities are low and connected to pH.

Conclusion

In this study, an RP-HPLC-DAD method was developed for the first time to determine CBZ and NTB concurrently in their dosage forms. The developed method was conducted to investigate the effects of both drugs on their solubility in binary mixture using different simulating body fluids at different pH. The results of solubility studies indicated that both drugs significantly changed the solubility of each other. Simplicity, rapidity, reliability, accuracy, low cost, high recovery, and sensitivity of the recommended method provide it an advantage over many other methods for simultaneous determination of NTB and CBZ. The proposed method can be used for analysis of these drugs in routine quality control laboratories.

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The authors declare no conflict of interest.

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