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A Simple LC-MS/MS Method for Determination of Vemurafenib in Rat Plasma Fed with High Fat Diet

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

In this study, high fat diet was fed to rats and the amount of vemurafenib in rat plasma was determined by the developed liquid chromatography with tandem mass spectrometry. The calibration curve was linear between 0.01 and 0.8 µg mL^{-1} vemurafenib with 0.999 regression coefficient. The limit of detection and quantification of the method are estimated from the signal to noise ratio 3:1 and 10:1, respectively. These are 1.10^{-4} µg mL^{-1} for LOD and 4. 10^{-4} µg mL^{-1} for LOQ. This method has been found to be reproducible and highly sensitive and provides a combination of faster analysis time and improved limits of detection.

Keywords: Vemurafenib; rat plasma; LC-MS/MS; fat diet.

1. INTRODUCTION

Vemurafenib (VEM, Fig. 1) is the first selective, potent and orally bioavailable inhibitor of the serine/threonine-protein kinase b-raf protein encoded by the V600E mutated braf gene [1]. Vemurafenib was recently approved by authorized agencies for the treatment of

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metastatic melanomas harboring BRAFV600E mutations [2]. A relationship between tumor size change and plasma exposure to VEM has been reported [3]. Mutation of the braf gene is very common in melanoma [4,5]. Recently, improved survival was observed in an ongoing Phase III clinical study with vemurafenib compared to dacarbazine in patients with previously untreated metastatic melanoma with this mutation [6]. Total metabolite levels in plasma were below 6% compared to the parent drug level [7]. To further improve survival of melanoma patients after a 40 years lack of progress, combination therapies will be explored, especially combinations with immunomodulators are expected to be very active [1].

The literature survey revealed that few liquid chromatography – tandem mass spectrometry (LC-MS/MS) methods have been reported for the analysis of vemurafenib in human and rat plasma [6-13].

The aim of the study, determination of vemurafenib amounts in rat plasma which fed with high fat diet by developed LC-MS/MS method. Also simple protein precipitation with acetonitrile could be done in order to quantify VEM in high fat diet rat plasma samples. The developed method is simpler, faster and cheaper than published methods using liquid-liquid extraction or solid phase extraction for sample preparation technique.

2. METHODOLOGY

2.1 Chemicals, Reagents and Animals

All chemicals were analytical reagent-grade. Vemurafenib was kindly donated by Roche Pharm. Ind. (Istanbul, Turkey). Acetonitrile (organic modifier) and dimethylsulfoxide (DMSO) and formic acid were purchased from Merck (Darmstadt, Germany). Milli-Q system (Millipore, Bedford, MA, USA) was used to get ultrapure water. Male Wistar Albino rats (2–3 months old; equal weight) were purchased from the animal breeding laboratories of Afyon Kocatepe University Experimental Animal Research and Application Center (Afyon, Turkey). All rats were housed under standard conditions of temperature (23°C ± 2°C), humidity, and 12-h dark–light cycle. The animals were feed standard rat feed supplied by Bil-Yem Ltd. (Turkey).

2.2 Equipment

For the analysis of the VEM using LC-MS/MS was carried out on Agilent 6460 triple quad mass spectrometer. This instrument was equipped with an electrospray ionisation (ESI), operating in positive mode and configurated scan mode monitoring. All solutions were degassed by ultrasonication (Sonorex, Bandelin, Germany).

2.3 Solutions

2.3.1 Preparation of standards and stock solutions

The stock solution of VEM (1000 µg mL^{-1}) was prepared in DMSO and stored at +4 ^{o}C. The working solutions to spike plasma samples were diluted from the stock solution with acetonitrile. The calibration standards at six concentrations level (0.01 and 0.8 µgmL^{-1}) were prepared with spiking of the blank plasma samples with the working standard solutions. The blank plasma samples were stored at -20^{o}C.

2.4 Animal Study

300-350 gram Wistar Albino rats were used for the study. These rats were acclimated to lab conditions for 7 days before being taken into the experimental process. The rats were feed high fat diet by three weeks. In order to provide a high fat diet as much as the rats' nutritional needs, butter was melted and added according to the ratio of 25 grams of butter to 100 grams of standard diet (Mis Butter-MilkMan Milk and Milk Products. Food Industry and Trade Inc.). It was mixed with a mechanical stirrer to allow the oil to suck and then allowed to cool. High-fat diet prepared fresh daily. During the experiment, it was observed that the animals were in compliance with this diet.

After feeding with high-fat diet, VEM (10 mg/kg) was injected to rat and blood samples were collected after 12 hours. After completion of the experimental phase, the rats were treated with 87 mg / kgi.m. Preanesthesia with xylazine administration was then performed at a dose of 13 mg / kgi.m. Ketamine was fully anesthetized. During the collection of plasma samples, after the anesthesia, the rib cage was opened and blood samples were taken intracardially with EDTA (Ethylenediamine tetra acetic acid) syringe for analysis. Immediately after this, cervical dislocation was performed and sacrification was

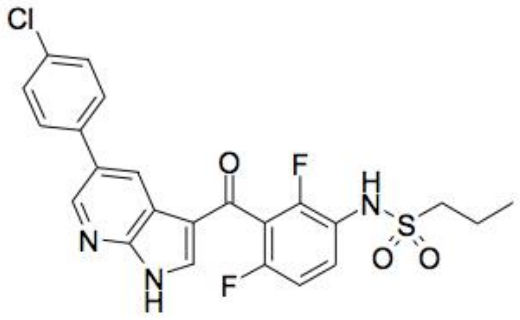
Compound	Chemical Structure
Vemurafenib N-{3-[5-(4-chlorophenyl)-1H-pyrrolo[2,3-b]pyridin-3-carbonyl]-2,4-difluorophenyl}propane-1-sulfonamide CAS No: 918504-65-1	 $C_{23}H_{18}ClF_2N_3O_3S$ MW : 489.9 g mol^{-1}

Fig 1. Chemical structure of vemurafenib

terminated. Plasma of the blood samples were separated by centrifugation at 3000 rpm for 10 minutes. Plasmas were collected in 1.5 mL Eppendorf tubes and stored at -80ºC until analysis.

2.5 Preparation of Rat Plasma Samples for Analysis

The proteins in the 500 µl rat plasma samples were crashed with 1000 µl acetonitrile. After vortexing for 3 minutes, the samples were centrifuged at 15000 rpm for 10 min. Finally, 1000 µl solution was transferred to a clean vial ran on LC-MS/MS system.

2.6 Chromatographic Conditions

For plasma sample analysis, ACN-water (50/50 (v/v) including % 0.1 (v/v) formic acid was used as a mobile phase. A Zorbax Eclipse plus C18 (2.1×50 mm ID ×1.8µ) column was used stationary phase. The column temperature was kept at 30 ^{0}C. Triple-quadrapole mass spectrometer with an electrospray ionization source has been used in positive mode. A mobile phase composed of ACN-water mixture containing 0.1% formic acid was used to attain good resolution, symmetrical peak shapes and shorter run time. Vemurafenib was monitored at *m/z* 490.0 → 383.0 and monitored at *m/z* 490.0 → 254.9. Table 1 summarizes the MS operating parameters.

3. RESULTS AND DISCUSSION

The calibration curves of vemurafenib were plotted peak areas of vemurafenib versus the spiked plasma concentrations at six levels (0.01, 0.05, 0.1, 0.2, 0.4 and 0.8 µg mL^{-1}). The calibration curve was linear between 0.01 and 0.8 µg mL^{-1} vemurafenib with 0.999 regression coefficient. The calibration curve equation is y = 626574x-1288, where x is the concentration and y is the peak area. LOD and LOQ of the method are estimated from the signal to noise ratio 3:1 and 10:1, respectively. These are 1.10^{-4} µg mL^{-1} for LOD and 4. 10^{-4} µg mL^{-1} for LOQ. Standard chromatogram of VEM was given in Fig. 2.

Table 1. Mass spectrometric parameters for the analysis of vemurafenib

Gas Temp (^{0}C)	325
Gas Flow (L/min)	11
Nebulizer (Psi)	45
Sheat gas heater (^{0}C)	400
Sheat gas flow	12
Capillary (V)	3000
Vcharging	500
Parent mass (m/z)	490
Product mass (m/z)	383
Dwell time (ms)	45
Collision energy (V)	30
Retention time (min)	1.150
Fragmentor voltage	160

Intra-day and inter-day analyses were utilized to investigate accuracy and precision of the developed method. Two different concentrations of vemurafenib (0.05 and 0.2 µg mL^{-1}) were analyzed six times in the same day and once in each day following to six consecutive days. The relative standard deviation (RSD) and the bias of intra- and inter-day studies were evaluated for accuracy and precision of the method (Table 2). The intra-day and inter-day results indicate that the developed method was accurate and precise.

The recovery of the developed method was investigated comparing the peak area obtained from spiked plasma samples with at same level

of working standard solutions (0.05 µg mL^{-1} vemurafenib). The recovery of vemurafenib from rat plasma samples was found to be 91.50% ± 0.60. The high recovery shows that the sample preparation step is efficient to retrieve VEM from plasma.

In the literature, when high-fat ketogenic diet (high fat, adequate protein and low carbohydrate) were given to xenograft rat, increase of serum acetoacetate levels and growing of human melanoma cells were observed [14]. In this study, in addition to the literature, the effects of high-fat diet on the amount of vemurafenib in rat plasma were investigated. In the literature, effects of high-fat diet on the rat plasma sample have not been investigated yet.

At the beginning of the study, the body weight of the rats was 345.9 ± 15.1 g and 358.1 ± 23.2 g after a 3-week high-fat diet. There was no statistical increase in the weight of rats despite high fat diet.

Rat plasma samples were collected 12 hours after injection of vemurafenib. Then, plasma samples were prepared as described in the 'Preparation of plasma samples for analysis'. Typical chromatograms of rat plasma sample is shown in Fig. 3. There are no extraneous peaks in chromatograms obtained for plasma samples. The amount of VEM (after 12 hours injection of drug) in rat plasma samples was calculated as 8.73 ± 0.052 (n=6).

The use of chemotherapeutic drugs in borderline or malignant obesity patients is an issue that needs to be considered when the fatty diet changes the efficacy of cancer drugs. It is obvious that a condition that decreases or inhibits the effectiveness of VEM , an important survival agent, will interfere with treatment. In our study, when the calculated values were compared with the literature values [6], we can say that the fat diet does not change the blood levels of the drug or the blood level of the drug is not affected by the fat diet.

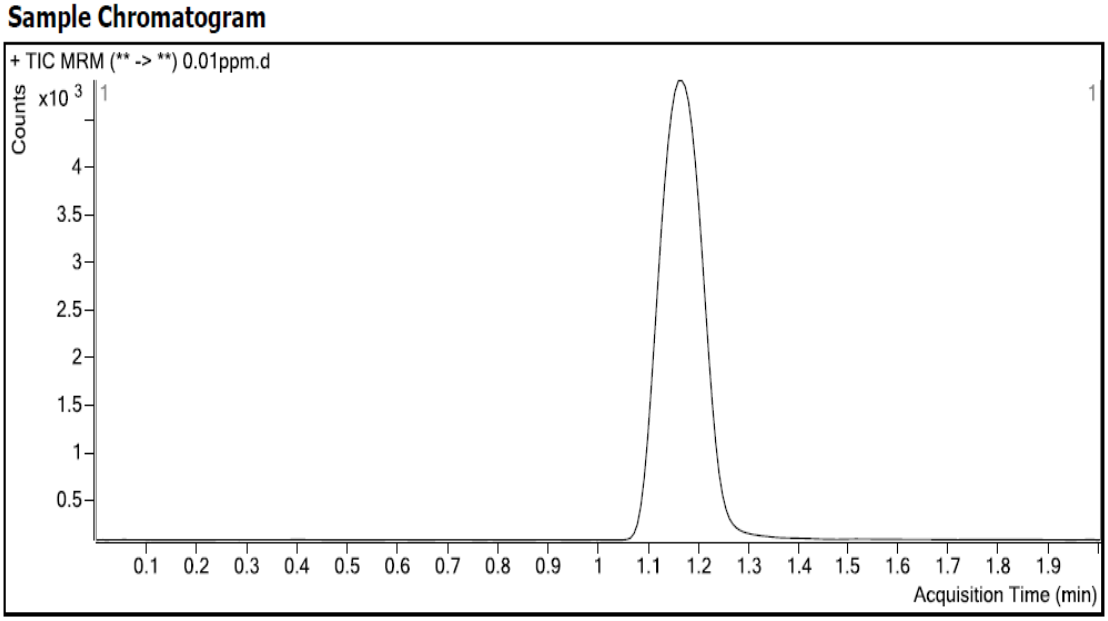


Fig. 2. Chromatogram of VEM standard (0.01 µg mL^{-1})

Table 2. Summary of repeatability (intra-day) and reproducibility (inter-day) precision data for vemurafenib by LC-MS/MS

Compound concentration (µg.mL^{-1})	Intra-day	Inter-day
	Mean Recovery* % ± RSD %	Mean Recovery* % ± RSD %
0.05	100.113 ± 0.638	100.027 ± 0.527
0.2	100.157 ± 0.514	99.998 ± 0.245

**Each value is obtained from six experiments (n=6)*

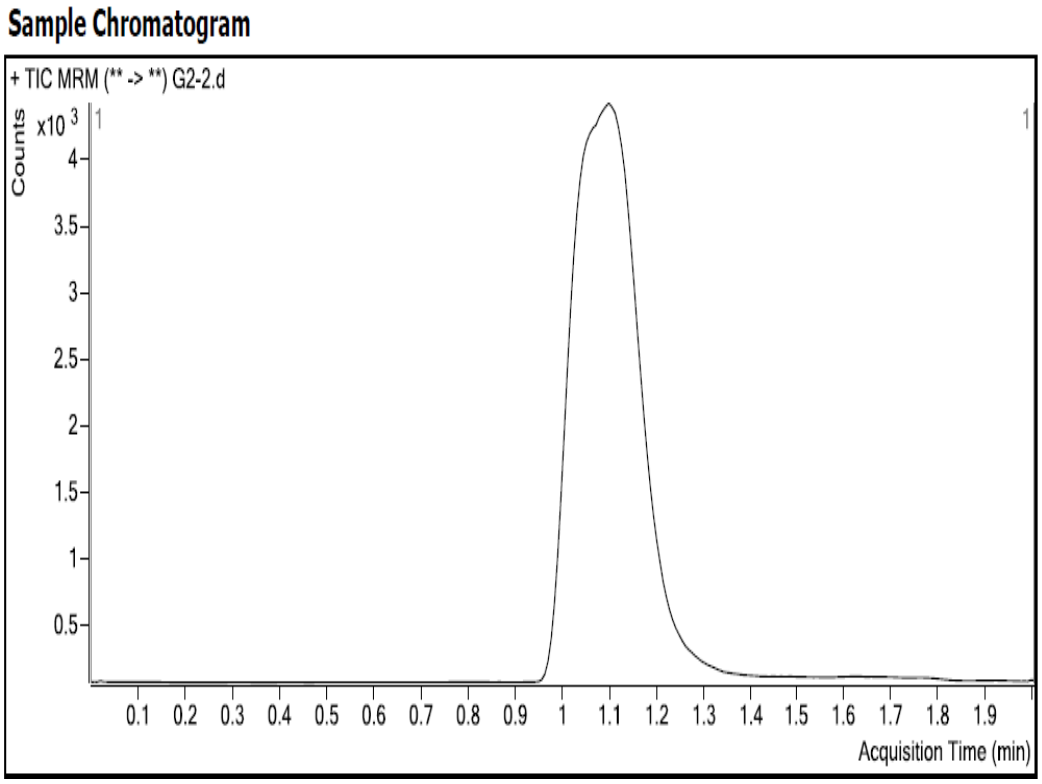


Fig. 3. Chromatogram of rat plasma sample after injection of VEM 12 hours later

4. CONCLUSION

Although several methods have been reported for determination of VEM, to the best of our knowledge, there are no data for VEM about high-fat diet on the rat plasma sample yet. Although the studies on the changes in the blood levels of high-fat diet VEM are limited, our study is promising to shed light on the studies evaluating the effects on human health.

Also in this work, simple, fast and effective LC-MS/MS method has been applied to determine VEM for rat plasma samples. Also, this method has been found to be reproducible and highly sensitive and provides a combination of faster analysis time and improved limits of detection.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The study protocol is approved by Animal Experimentation Local Ethics Board (No: AKUHAYDEK-38-16).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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