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Research Article

DETERMINATION OF VALPROIC ACID BY HPLC IN HUMAN BLOOD AND ITS RELATIONSHIP TO THERAPEUTIC DRUG MONITORING

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ABSTRACT
A novel HPLC method for the determination of valproic acid (VPA) in human serum using nonanoic acid as internal standard (I.S.) is described in this article. The eluates were separated with a reverse phase column of the dimensions C18 250 × 4.6 mm internal diameter maintained at a temperature of 48 °C. A mobile phase consisting of acetonitrile and 29mM phosphate buffer (pH 3.5) 49: 51 v/v was used at a flow rate of 1.2 ml/min. Wavelength used was 220 nm during valproic acid retention. The method was linear over a concentration range of 10 to 100 µg/ml for valproic acid. Recovery was greater than 93% over a concentration range of 20 to 100 µg/ml respectively. The retention time for valproic acid and Nonanoic acid was 9.7 and 13.8 minutes. The method is simple, rapid, accurate and sensitive and can be used for Therapeutic Drug Monitoring (TDM) in epileptic patient population.

Key Words: *HPLC, Valproic Acid, TDM, Mobile Phase, Phosphate Buffer*

INTRODUCTION
Valproic acid (2-propyl pentanoic acid, VPA; is a broad-spectrum antiepileptic drug with unique anticonvulsant properties and is used in the treatment of primary generalized seizures, partial seizures and myoclonic seizures as well as bipolar disorder (Simon and Penry, 1975; Mattson *et al.,* 1978). Several analytical methods are reported in scientific literature for the quantification of VPA in biological matrices (Cheng *et al.,* 2007). Earlier, VPA was analyzed by gas chromatography (GC) with flame ionization detector or by an immunological assay. GC analysis is difficult and it is required to extract the drug with an organic solvent and immunological techniques are expensive in a developing country context (Ram *et al.,* 1979). There are also LC-MS based techniques now available for several drugs including the VPA (Jain *et al.,* 2007). VPA is a branched chain carboxylic acid and attachment of a suitable chromophore or fluorophore to the carboxylic acid is necessary and has poor UV absorption. Therefore, we developed a simple method, which is rapid, accurate and sensitive for estimation of VPA on C18 column and evaluation of its performance for monitoring drug levels in epileptic patients.

MATERIALS AND METHODS
Sample Preparation and Extraction
Chemicals and Reagents

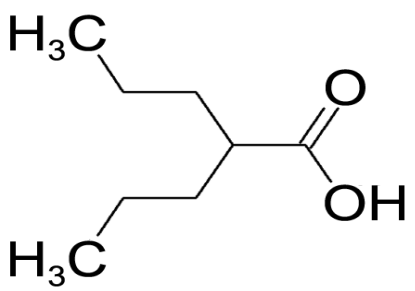

Figure 1: Chemical structure of Valproic acid

Valproic acid (Sodium valproate) and Nonanoic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals and solvents for e.g. acetonitrile, methanol and phosphate buffer were of analytical and HPLC-grade and were obtained from (Fisher Scientific, USA). De-ionized water was obtained from a milli-Q apparatus and was used in this study.

Extraction Procedure

To 200 μl serum samples, the acetonitrile solution of nonaoic acid equivalent to 2.0 mg was added as internal standard and shaken well. Then an equivalent amount of (200 μl) acetonitrile was added for protein precipitation and mixed on a cyclomixer for 1 min and centrifuged at 5000 rpm using a REMI centrifuge (R8C laboratory centrifuge, REMI motors, Mumbai, India) for 10 minutes. 20 μl of the supernatant were injected into the HPLC column.

Preparation of Internal Standard

50 mg of Nonanoic acid was weighed for making the working standard and transferred into a 50 mL volumetric flask with about 25 mL of acetonitrile and make the volume to 2.0 mg/mL concentration solution. Correction was done for the final concentration of nonanoic acid showing its potency and actual weight. It was stored as the stock solution in the refrigerator at 4-8 ^{0}C.Acetonitrile was used to dilute the solution for experimental work.

Apparatus & Chromatographic Conditions

The HPLC system consisted of a model LC–20AP chromatograph (Shimadzu, Japan), a model DGU-14A de-gasser (Shimadzu, Kyoto, Japan), a model SIL–10ADvp auto injector (Shimadzu, Kyoto, Japan). Separation was achieved on C-18 column of 250 x 4.6 x 5 µm specifications (Beckman Coulter, U.S.A) and detected with UV-VIS detector model SPD-20A (Shimadzu, Kyoto, Japan) at wavelength 220 nm. The part of extraction consisted of a model 2601 multi – tube vortexer (Scientific Manufacturing Industries, U.S.A), a model Z 383 K centrifuge (Accurate Scientific Instruments, India) and a model 4322100 vortex – evaporator (Buchler Instruments a Labconco Company). The mobile phase consisted of acetonitrile – 29m M phosphate buffer (49:51). It was filtered with 0.25 µm membrane filter (Agilent Technology, Singapore) before use. Chromatography was performed at 48 ^{0}Ctemperature. Flow rate was 1.2 ml/min and injection volume was 20 µl.

Recovery and Accuracy

The total recovery from the serum samples was estimated by comparing the amount of valproic acid from serum samples with that of recovery standards, which were processed similarly without serum matrix using methanol. The accuracy of the procedure was determined by expressing the mean calculated concentration as a percentage of the spiked concentration.

Discussion

VPA is indicated as monotherapy for epileptic seizures like the myoclonus, partial and tonic-clonic seizures, and myoclonic juvenile epilepsy (Paulo *et al.,* 2013; Levisohn and Holland, 2007). However, some systemic toxicity has been observed. VPA is also used as an alternative to lithium in patients with bipolar disorder, as well as in depression, migraine, febrile convulsions. Thus determining the blood samples is critical to understand the pharmacokinetic profile of the drug in a patient and also for therapeutic drug monitoring purpose (Tyagi, 2012). In the GC-MS technique carboxylic acids can be converted to their phenacyl esters, and alpha-bromoacetophenone is added to the organic extract before evaporating the solvent. These esters are relatively less volatile than the acids themselves and the extracting solvent can be removed without any loss of valproic acid or internal standard. The phenacyl esters, when chromatographed on 3% OV-17, produce sharp, well-shaped peaks and show high response for the flame ionization detector. A selective ultraperformance liquid chromatographic (UPLC) method for the quantification of valproic acid and its known related impurities using ion pair reagent has been developed (Thakkar *et al.,* 2012).

However using the HPLC method, VPA is well separated from the internal standard, from reagents and plasma constituents, and from some commonly prescribed drugs. In our study, the total recovery was in the range of 93.23 to 95.11 %. The retention time for valproic acid and Nonanoic acid was 9.7 and 13.8

minutes (Kondo *et al.,* 1985). The intraday precision of the assay was determined by analyzing five spiked serum samples at each concentration on the same day. Inter day precision was determined by analyzing spiked serum samples on five different days. The inter-day relative standard deviation (RSD) ranged from 0.512-2.261, 0.639–3.032, 0.859–3.568, 2.325–4.179 and 0.840–3.452 for 10, 20, 40 and 80 and 100 µg/ml respectively. The intra-day RSDs were 4.732, 4.340, 2.659, 2.332 and 1.577 for 10, 20, 40, 80 and 100 µg/ml respectively. These values were within the limits (<15%) specified for inter day and intra day precision. The recovery from serum was estimated at 10, 20, 40, 60, 80 and 100 µg/ml concentrations. Proteins in serum samples (in six replicates) containing VPA and internal standard were precipitated and analyzed. Six samples containing similar concentration of VPA in methanol were directly injected and peak areas were measured. Absolute recovery was calculated by comparing the peak areas for direct injection of pure VPA with that obtained from serum samples spiked with the same amount of VPA and processed similarly. The absolute recoveries ranged from 93.23–95.11% (Table 1). This technique used is precise and cost effective and is as efficient as other techniques described elsewhere (Kishore *et al.*, 2003). The accuracy of the method was verified by comparing the concentrations of VPA measured in spiked serum with the actual concentrations added.

In conclusion, it can be stated that this validated method permits the rapid, efficient and robust analysis of VPA. Validation demonstrates that the method permits the reliable and unambiguous identification and quantification of VPA. The method also exhibits satisfactory selectivity, linearity, precision i.e repeatability and also intermediate precision, accuracy, and recovery.

Table 1: Total recovery and accuracy of estimation of valproic acid in human serum

Nos.	Concentration µg/ml	Total Recovery (%)	Accuracy (%)
1)	10	93.23 ± 1.61	95.38 ± 1.11
2)	20	94.41 ± 2.38	95.27± 1.34
3)	40	93.52 ± 2.65	94.29 ± 1.47
4)	80	95.11 ± 2.11	98.63 ± 2.07
5)	100	94.47 ± 2.63	97.43 ± 2.15

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