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Determination of VPA and its Two Important Metabolites in Iranian Overdosed Patients

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Abstract: In this study, we determined and evaluated the correlation between the dose of Valproate (VPA) and the concentrations of VPA and two important metabolites (2-ene-VPA and 4-ene-VPA) in serum of intoxicated patients referred to Loghman hospital. VPA poisoning is an increasing clinical problem. 2-ene-VPA is neurotoxic and the 4-ene-VPA has a key role in hepatotoxicity of VPA. Following clinical evaluations, two blood samples at referring time (time zero) and 12 h later were collected from 19 intoxicated patients. VPA and its metabolites were extracted from serum samples and measured using Gas chromatography-mass (GC-MS) spectrometry analysis. There was a correlation between VPA dose and concentration of VPA in serum of intoxicated cases at referring time ($p<0.01$). Significant reduction between mean serum concentration of VPA and metabolites was observed after 12 h (For VPA $p<0.05$, for 2-ene-VPA and 4-ene-VPA $p<0.01$). Evaluation of serum levels of VPA metabolites can provide information about potential risk of side effects and better management of patients.

Key words: 2-ene-VPA, 4-ene-VPA, analysis, hepatotoxin, neurotoxin

INTRODUCTION

Valproic Acid (VPA) is a widely used drug in the treatment of epilepsy and bipolar disorder as well as prophylaxis and termination (counteraction) of migraine headache (Citrome *et al*., 1998; Garnie *et al*., 1982). The increased use and availability of VPA in the treatment of patients with psychiatric and neurological disorders has led to a steady rise in the number of VPA overdoses cases (Wilimowska *et al*., 2006). VPA is a week acid with the volume of distribution between 0.1-0.5 L kg^{-1}. The therapeutic concentration of VPA ranges between 50-100 μg mL^{-1}. Serum concentration <450 μg mL^{-1} causes limited toxicity, however, levels greater than 850 μg mL^{-1} create serious intoxication (Graudins and Aaron, 1996; Sztajnkrycer, 2002).

After therapeutic dosing, VPA is usually absorbed rapidly from the gastrointestinal tract and peak concentrations are recorded at 2 to 3 h. In overdose, delayed peak levels have been recognized (Graudins and Aaron, 1996). At normal serum levels, VPA is extensively (≥90%) bound to plasma proteins. The extent of binding decreases to 81.5% at 130 μg mL^{-1} and only 35% of the drug is protein bound at levels 300 μg mL^{-1} (Klotz and Antonin, 1977; Kane *et al*., 2000).

In overdosing, the most common symptom is the central nervous system depression, which may, progress to coma and respiratory system depression. It has been speculated that the increased free drug fraction in overdose allows for greater central nervous system penetration and toxicity (Dupuis *et al*., 1990). Pancreatitis occurs both during long-term therapy with VPA and

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acute overdose. In contrast, hepatotoxicity has rarely been reported in connection with acute overdose (Dupuis *et al*., 1990; Sztajnkrycer, 2002).

VPA is extensively metabolized by the liver and produce biologically active metabolites (Davis *et al*., 1994). VPA is metabolized via ß-oxidation and produces 2-propyl-2 pentenoic acid (2-ene-VPA). This metabolite has anticonvulsant efficacy and is persistent in brain. It has been reported that 2-ene-VPA is neurotoxic and may result in cerebral edema (Dupuis *et al*.,1990; Loscher and Nau, 1982).

2-propyl-4-pentenoic acid (4-ene-VPA), another monounsaturated metabolite of VPA, is formed via a distinct microsomal cytochrome P-450-mediated desaturation reaction (Rettenmeier *et al*.,1987). 4-ene-VPA is similar to methylene cyclopropyl acetic acid, the hypoglycin A metabolite responsible for Jamaican vomiting sickness and 4- pentenoic acid, a hepatotoxic agent, which produces a Reye-like syndrome in rats (Cotariu and Zaidman, 1988; Suchy *et al*., 1979).

VPA inhibits many hepatic enzyme system activities involved in drug metabolism to a different extent and is able to significantly displace drugs from plasma albumin. When administrated with other drugs, the inhibitory activity of VPA can produce some harmful effects (Fowler, 1978; Riva *et al*., 1996).

Clinical treatment in VPA overdosing is mainly supportive with application of basic toxicological principles. Decontamination should be performed within one hour of ingestion, or when ingestion of sustained release preparation is probable. Despite the high protein binding of 90-95%, because of saturation of protein binding sites, enhanced elimination methods have been implied, resulting in increased fraction of unbound VPA (Franssen *et al*., 1999; Meek *et al*., 2004).

Because of the its vast usage and high incidence of poisoning with this drug, the recent study was designed with the aim of investigating the relationship between intoxication and serum concentration of VPA and two important metabolite of this drug in patients referring to the poisoning ward of Loghman hospital, the referral poisoning center in Iran.

MATERIALS AND METHODS

Patients: Following approval of the ethics committee of Tehran University of Medical sciences, 19 patients intoxicated with VPA between September 2006 to March 2007 was entered to this study. All patients (13 female and 6 male, their age varied between 17 to 30 years) had committed suicide and were admitted to the emergency of Loghman hospital with suspected VPA overdose. Two blood samples were collected from patients at the time of admission and 12 h later to measure the serum concentration of VPA, 2-ene-VPA and 4-ene-VPA.

Detailed information about ingested drugs as well as selected information about the clinical condition of the 19 poisoned patients are shown in Table 1.

Chemicals: As standard for metabolite measurements, the 2-ene-VPA and 4-ene-VPA were synthesized in Faculty of Pharmcy according to reported methods (Bojic *et al*., 1996; Schafer, 1990). We purchased VPA from Sigma Chemical Company (St. Louis, USA), Octanoic acid and BSTFA (N,O-bis[Trimethylsilyl]trifluoroacetamide) from Fluka (Chemical Corp, Switzerland). Ethyl acetate and Perchloric acid were obtained from Merck (Darmstadt, Germany).

Methods: 0.5 mL of each serum sample was drawn into a disposable 1.5 mL Eppendorf microtube and spiked with 20 μL; 2.5 μg mL^{-1} octanoic acid as internal standard (I.S.). Then 50 μL of 12% Perchloric acid and 1 mL of ethyl acetate were added. The tube was shaken for 15 min and then centrifuged for 10 min at 10000 rpm. An 800 μL portion of the supernatant organic phase was transferred to a 1.5 mL disposable glass reaction vial and evaporated under a nitrogen stream at 45°C. After addition of 50 μL BSTFA to the residue, the reaction vial was mixed thoroughly using vortex mixer followed by heating at 60°C for 30 min. The resulting solution was analyzed by GC-MS.

The instrument used for GC-MS analysis was an Agilent (Agilent Technologies, Palo Alto, CA, USA) 6890 plus gas chromatograph equipped with a 5937 mass selective detector quadrupole mass spectrometer. A 1 μL aliquot of the final derivatized extract was injected into the system operated in the split-less mode. The injector temperature was set at 250°C. The column was an HP-1 cross-linked methyl siloxane, 60 m×0.25 mm i.d., film thickness 1 μm (Agilent Technologies, Palo Alto, CA, USA). The GC oven temperature was initially set at 50°C hold for 0.5 min and then programmed to 280°C at a rate of 20°C min^{-1} and maintained for 3 min. The temperature of the transfer line was maintained at 280°C. Helium (99.999%) was used as carrier gas at 1 mL min^{-1}. The source and quadrupole temperatures were kept at 230 and 150°C, respectively. The electronic beam energy of the mass spectrometer was set at 70 eV. The mass selective detector was operated in Electron Impact (EI) mode using Selected Ion Monitoring (SIM). The dwell time of each ion was set at 100 ms. The ions monitored were m/z 199 for 2-ene-VPA and 4-ene-VPA and m/z 201 for VPA and octanoic acid. The GC conditions were selected to

Table 1: History and manifestations of 19 Valproate-overdosed patients, admitted to I.C.U, in Loghman hospital, from September 2006 to Mars 2007

Patient ID No.	Sex	Age (year)	Dose mg kg^{-1}	Time after ingestion (h)	History of treating with VPA	Symptoms	Other drugs	Duration of hospitalization (day)	Treatments
1	M	20	Unknown	6	3 months	Somnolent	No	3	Symptomatic treatment, Activated charcoal
2	F	23	150	5	8 years	Somnolent, stable haemodynamically	No	4	Symptomatic treatment, Gastric washing, Activated charcoal
3	F	27	80	2	no	Somnolent, Mydriasis	No	3	Symptomatic treatment Activated charcoal
4	F	19	Unknown	3	1 month	Somnolent, convulsions	unknown	3	Symptomatic treatment Activated charcoal
5	F	17	38	1	8 months	Unconscious	Mix	4	Ventilation, NG tube, Gastric washing, Activated charcoal
6	F	24	57	1	no	Somnolent, headache, vomiting	No	3	Symptomatic treatment Activated charcoal
7	M	19	218	2	3 years	Somnolent, nausea, decreased blood pressure	Imiperamin clonazepam	4	NG tube, Gastric washing Activated charcoal
8	F	17	Unknown	1	no	Somnolent, Gastric pain, 12 breaths min^{-1}	No	3	Symptomatic treatment Activated charcoal
9	F	30	124	1	4 years	positive reaction to light, Unconscious, mydriasis	Phenobarbital	5	Symptomatic treatment, O2 therapy, gastric lavage, Activated charcoal
10	F	25	138	8	7 months	Somnolent, hypotension 100/60 mm Hg, blood hemolysis, Headache	Unknown	4	Symptomatic treatment O2therapy, Activated charcoal
11	M	24	87.50	6	3 months	Hypotension(80/pulse), nystagmous, positive reaction to light	Properanolol carbamazepine?	3	Symptomatic treatment O2therapy, Activated charcoal
12	M	24	55	8	2 weeks	hypotension (100/60), Somnolent, vometing	Alperazolam serteralin	4	Symptomatic treatment Activated charcoal
13	F	22	70	4	9 months	Coma, hypotension (100/60)	Tricyclic antidepressant+doxepin	3	Intubation and Ventilation, Activated charcoal
14	F	28	163	3	4 years	Somnolent, Miosis, 12 breaths min^{-1}	Clonazepam phenobarbital	4	Symptomatic treatment, Activated charcoal
15	F	28	62	2	2 years	Somnolent, vometing	imiperamine	6	NG tube, Gastric washing Activated charcoal
16	M	18	74	7	9 months	Lethargy, drowsiness	mix	4	Symptomatic treatment, Activated charcoal
17	F	26	Unknown	5	1 year	Vomiting, positive reaction to light	no	3	Symptomatic treatment, Activated charcoal
18	M	18	274	6	no	Coma, PTTÔ	Biperidin Clonazepam olanzapin	5	Intubation and ventilation, Activated Charcoal, Hemodialysis
19	F	20	50	5	2 years	Somnolent	mix	2	Symptomatic treatment Activated charcoal

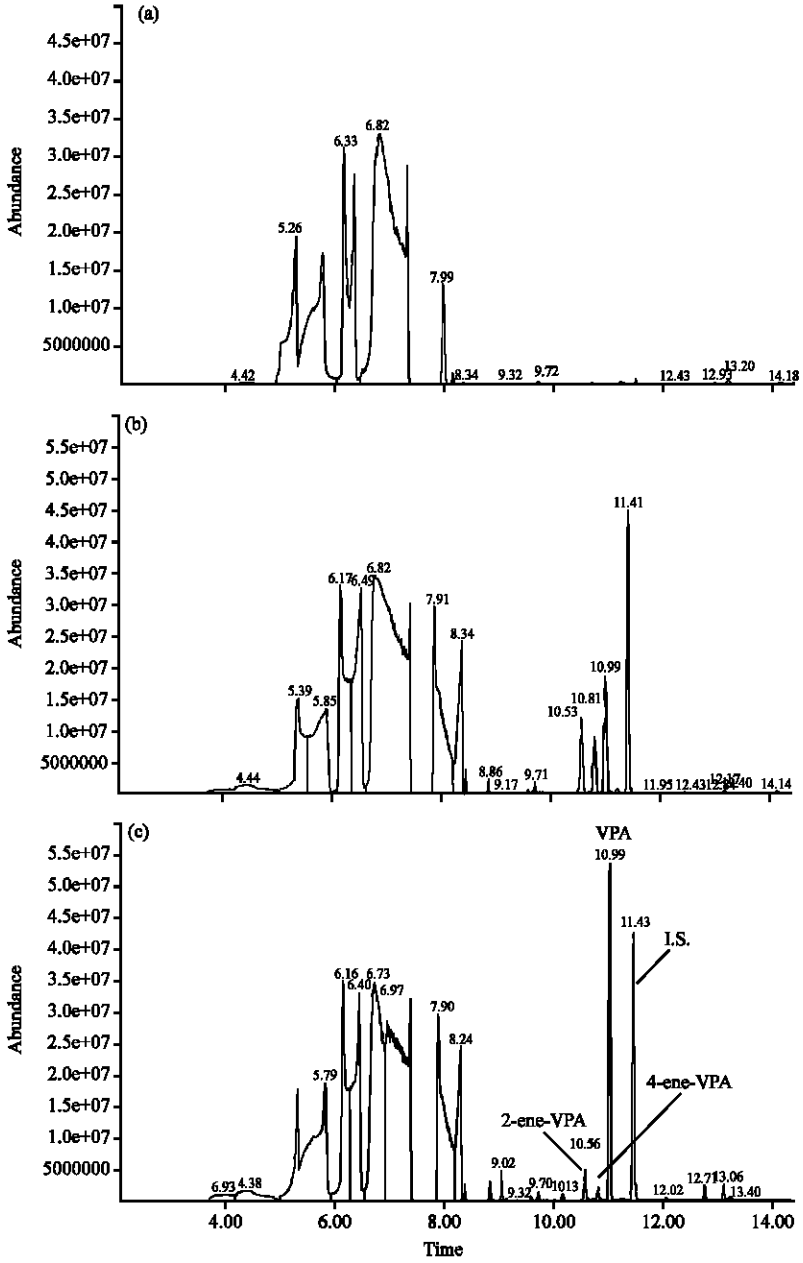

Fig. 1: Representative chromatograms of (a) blank, (b) spiked and (c) real plasma samples analyzed by GC-MS after extraction and derivatization

Table 2: Selected ions used for detection of target analytes by GC-MS in SIM mode

Ion groups	Analyte	Molecular ion	Selected ions	Confirmed ions
		----------	(m z^{-1})	----------
1	VPA	216	201	174,145
2	4-ene-VPA	214	199	185, 172
2	2-ene-VPA	214	199	185, 169
1	octanoic acid	216	201	145,117

minimize the time of analysis while allowing all the analytes to elute in acquisition groups containing suitable number of ions for monitoring (Table 2). Chromatograms of spiked, blank and real samples monitored for VPA and target metabolites were shown in Fig. 1a-c.

Quantitation: For constructing the calibration curves and making quality control samples (QC), a pool of plasma taken from healthy volunteers was used as blank matrix. This matrix is routinely used in our laboratory as blank and preliminary study showed its suitability for the

purpose of the present study. Quantitation of the VPA and metabolites in each sample was performed by calibration line method, using octanoic acid as internal standard based on peak areas. Calibration curve was constructed by adding fixed amount of I.S. solution (20 μL; 2.5 μg mL^{-1} in ethyl acetate) and varying quantities of the analyte standards to 0.5 mL of each sample to make the final concentrations range of 25-150 μg mL^{-1} for VPA,0.25-8 μg mL^{-1} for 2-ene-VPA and 5-200 ng mL^{-1} for 4-ene-VPA. The samples were then extracted and derivatized as described above.

Statistics: SPSS 16 for Windows was used for statistical analysis. The data presented as Mean±SD. Statistical analysis was performed using bivariate correlation test and paired t-test. The $p<0.05$ was considered statistically significant.

RESULTS

Mass spectrometric properties of derivatized analytes: The mass spectra of the analytes derivatives are shown in Fig. 2a-c. The mass spectra are dominated by the daughter ions that have lost one methyl group. Identification of daughter ions was annotated on the mass spectra in Fig. 2a-c. Each daughter ion shows a group of peaks representing the natural isotope abundance of the Si element. 2-ene-VPA and 4-ene-VPA show similar fragmentation patterns.

Quantitative analysis: The calibration curves parameters shown in Table 3 were obtained under the optimized conditions. Linearity of calibration curves were determined in the range of 25-150 μg mL^{-1} for VPA, 0.25-8 μg mL^{-1} for 2-ene-VPA and 0.005-0.2 μg mL^{-1} for 4-ene-VPA. Coefficient of correlation ranged from 0.994 to 0.998. Limit of detection (LOD) calculated as three times the baseline noise (S/N = 3) after 5 successive extractions of blank sample. According to the ICH (International conference on harmonization of technical requirements for bioanalytical methods) criteria for analytical method validation, limit of quantification (LOQ) for each analyte was determined as the lowest concentration on the calibration curve with a precision of less than 20% Coefficient of Variation (CV) and an accuracy of 80 to 120%. The estimated recoveries at three different concentration levels were shown in Table 4. Recovery studies were performed by spiking the analytes standards in a blank plasma sample at 30, 80, 130 μg mL^{-1} for VPA, 0.3, 1.5, 6 μg mL^{-1} for 2-ene-VPA and 0.0075, 0.040, 0.150 ng mL^{-1} for 4-ene-VPA (n = 6). Internal standard was added just before derivatization and the ratios of the most abundant ion of the analytes to that of internal standard were calculated. These values were compared with the ratios obtained for reference QC extracts, which were prepared by adding the same amounts of the analytes standards to the final plasma extract.

Clinical results: In 19 patients (68% female and 32% male) with the age between 17-30 (22.57±4.07) the mean time after ingestion of VPA and referring to ICU was 3.8 h. Fifteen cases (78.9%) had a history of epilepsy or mood disturbances and were under treatment with VPA alone or in combination with psychotropic drugs. Four cases (21%) had ingested unknown amount of drug, the mean dose of VPA in 15 cases were 109.3±68.22 mg kg^{-1}.

The mean of serum concentrations of VPA and two metabolites at referring time and 12 hours after hospitalization is summarized in Table 5. Mean serum concentration of VPA, 2-ene-VPA and 4-ene-VPA were significantly reduced after 12 h ($p<0.05$ for VPA, $p<0.01$ for 2-ene-VPA and 4-ene-VPA).

There was a correlation between dose and serum concentration of VPA at referring time ($R^2 = 0.577$, $p<0.01$).

Table 3: Calibration lines parameters of the developed method for determination of VPA and its metabolites in human plasma samples

Compound	Linear range (μg mL^{-1})	LOQ (μg mL^{-1})	Calibration line equation	Square of the correlation coefficient (r^2)
VPA	25-150	25	y = 0.010x + 0.005	0.998
2-ene- VPA	0.25-8	0.25	y = 0.006x-0.0009	0.998
4-ene- VPA	0.005-0.2	0.005	y = 0.0127x-0.0723	0.994

Table 4: Estimated recoveries and method precision for target analytes at different concentrations (n = 6) in QC samples

Compounds	Sample	Nominal conc. (μg mL^{-1})	Mean of Calc. Conc.[a] (μg mL^{-1})	CV% of Calc. Conc.	RE % of Calc. Conc.[b]	Estimated recoveries (%)	CV% of Calc. recovery
VPA	QC1	30.0000	26.2000	10.2	13.0	84	12.2
	QC2	80.0000	84.0000	8.7	5.0	86	12.6
	QC3	130.0000	126.0000	8.3	3.0	85	10.5
2-ene-VPA	QC1	0.3000	0.2700	10.6	10.0	88	11.5
	QC2	1.5000	1.6200	9.4	8.0	84	11.4
	QC3	6.0000	6.1000	7.5	1.6	83	12.4
4-ene-VPA	QC1	0.0075	0.0065	11.2	13.3	86	12.6
	QC2	0.0400	0.0430	9.5	7.5	82	11.3
	QC3	0.1500	0.1550	8.6	3.3	87	12.5

[a]Calculated concentration; [b]Relative error = [1-(Calculated Conc./Nominal Conc.)]×100

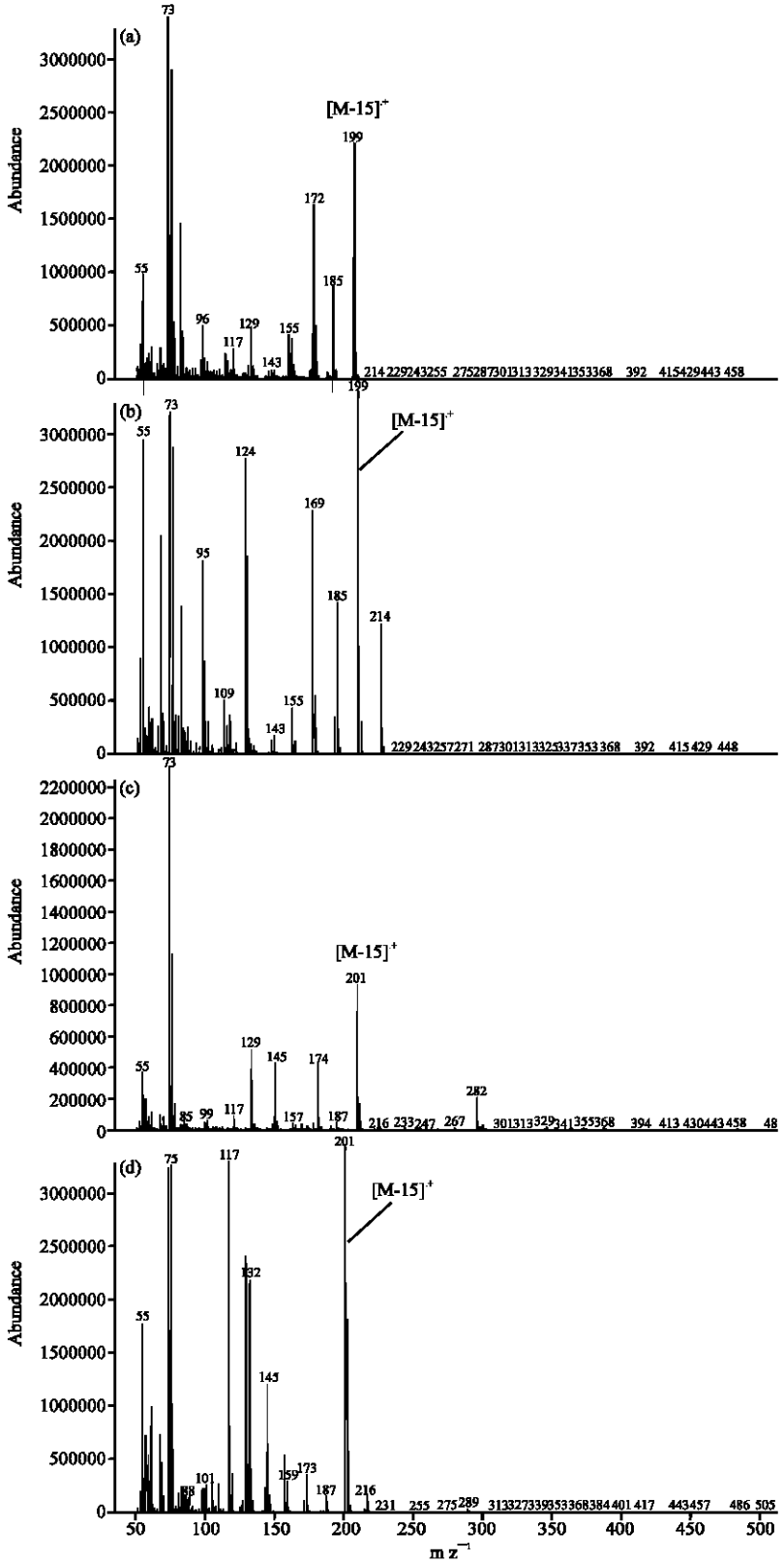

Fig. 2: Mass spectra of TMS- derivatives of (a) 4-ene-VPA, (b) 2-ene-VPA, (c) VPA and (d) octanoic acid

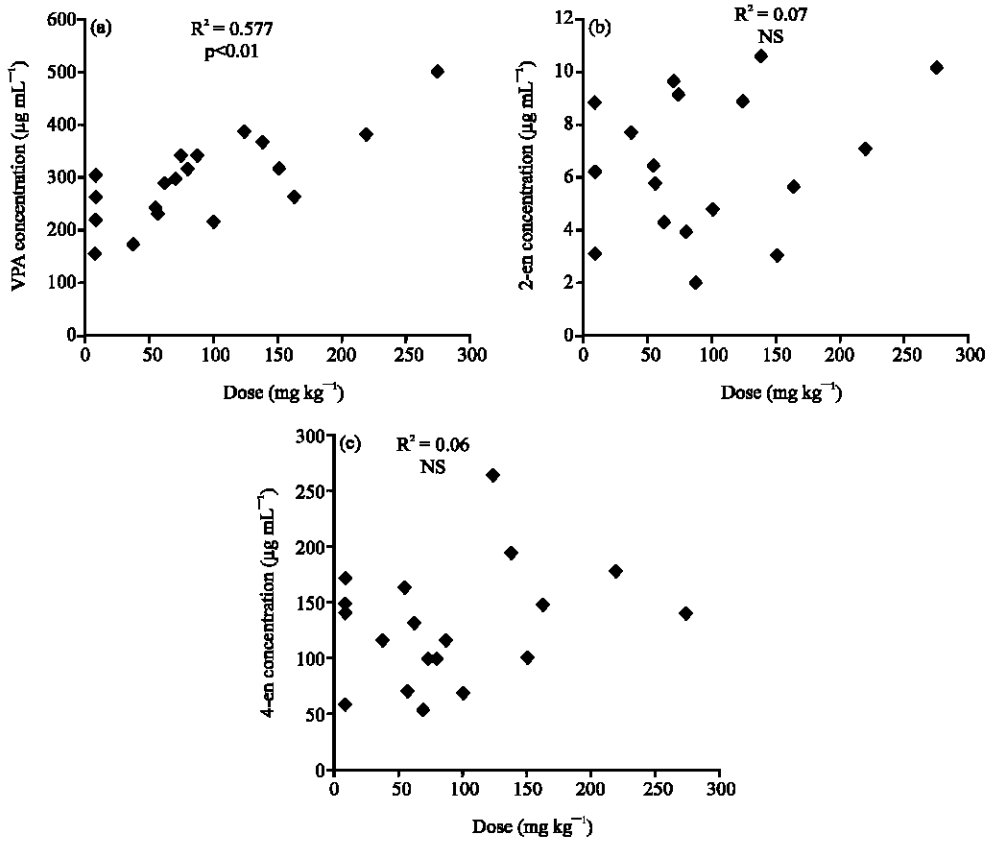

Fig. 3: Correlations between dose (mg kg^{-1}) and serum level of (a) valproate (VPA) (b) 2-ene-VPA (c) 4-ene-VPA. (a) correlation between dose and VPA concentration, (b) correlation between dose and 2-ene-VPA concentration and (c) correlation between dose and 4-ene-VPA concentration

Table 5: Serum concentrations of valproate and its metabolites. Data in the table are expressed as Mean±SD

Concentration	At referring time	12 h later
VPA (μg mL^{-1})	293.8±82.68	268.3±65
2-ene-VPA (μg mL^{-1})	6.5±2.57	5.8±2.53
4-ene-VPA (ng mL^{-1})	129.3±52.37	114.0±51.46

No significant correlation was observed between VPA dose and 2-ene-VPA or 4-ene-VPA (Fig. 3a-c). The average duration of hospitalization was 88.3±8 h.

DISCUSSION

Quantitative analysis: The precision of the method was evaluated in terms of repeatability (or interday precision) by calculation the analytes concentration in quality control samples, which prepared at three levels (each six replicates) on three consecutive days. Interday precision values for all analytes were always <15%. Expression of the intraday precision are the coefficients of variation (CV%) of determined responses of six replicates of Quality Control (QC) samples, which were prepared at three levels and reported in Table 4. All these results indicate the feasibility and reliability of the developed method for determining the VPA and its two important metabolites in human plasma samples.

Clinical findings: In this study we have evaluated the relationship between dose and serum concentration of VPA and metabolites in Iranian overdosed patients.

There was a correlation between dose and serum concentration of VPA at referring time, while no significant correlation was observed between VPA dose and 2-ene-VPA or 4-ene-VPA. It seems that the time between ingestion and first bleeding wasn't adequate for elevation of metabolites.

In acute overdose, VPA toxicity causes lethargy and drowsiness (Spiller *et al*., 2000). Considering the clinical symptoms, the most common complications was somnolent. 4 cases had vomiting and 1 patient complained from drowsiness and lethargy. This is consistent with Spiller *et al.* (2000) and Isbister *et al.* (2003) that concluded VPA causes mild toxicity in the majority of cases.

In Isbister *et al.* study (2003) drowsiness occurred in 2 patients taking more than 200 mg kg^{-1}, however, our patient with drowsiness had ingested 74 mg kg^{-1} VPA.

Severe overdose has been associated with respiratory failure, renal failure, acute pancreatitis, central nervous system depression, hyperammonaemia, hepatotoxicity, leucopenia, thrombocytopenia,metabolic acidosis and hypothermia (Seger, 1998; Robinson and Abbott, 2005; Tohen *et al*., 1995).

Hepatotoxicity may be asymptomatic with elevated serum liver enzymes (Ware and Milward-Sadler, 1980). 4-ene-VPA has been implicated in hepatotoxicity; however, cases of hepatotoxicity have been rarely reported with acute overdose (Graudins and Aaron, 1996).

The condition of our patients corresponded to the VPA and other drug concentrations.

Massive over doses greater than 400 mg kg^{-1} can cause severe toxicity (Isbister *et al*., 2003). None of our cases had ingested this amount of VPA. Most of them were multiple drug exposures, so, significant clinical effects were related to mixed toxicity (e.g., hypotension and coma).

Peak concentrations usually occur within 2 to 3 for syrup, capsules and uncoated tablets (Perucca, 2002). In Spiller *et al*. (2000) study the maximum measured VPA was 1840 μg mL^{-1}.

Wilimowska *et al*. (2006) reported the highest concentration of VPA (684 μg mL^{-1}) 3 h after ingestion. The clinical condition of patient was moderate, but the activity of aminotransferases increased. In our cases maximum concentration of VPA was 500 μg mL^{-1} observed 3 h after ingestion and she had normal aminotransferases.

Only one case had elevated aminotransferases whereas his 4-ene-VPA serum concentration was at the level of other cases (100.06 ng mL^{-1} in first sampling and 84.43 ng mL^{-1} after 12 h), he was addict with the history of daily tramadol use. The toxic serum level of 4-ene-VPA might exist >500 ng mL^{-1}, however in our study the maximum serum level of 4-ene-VPA was 263 ng mL^{-1} (Kondo *et al*.,1992).

In therapeutic dose of VPA, plasma concentration of 2ene-VPA ranges from 1-10 μg mL^{-1} that is less than 20% of parent drug (Levy *et al*., 2002).

2-ene-VPA may mediate cerebral edema and may be responsible for prolonged coma. Patients have been noted to be comatose with normal serum VPA concentrations, a finding attributed to the presence of unmeasured metabolites, such as 2-ene-VPA (Sztajnkrycer, 2002).

The mean concentration of this metabolite wasn't high in our cases (6.5±2.57 μg mL^{-1}) and the maximum ratio was related to 10th case without any unconsciousness or brain edema (10.57 μg mL^{-1}).

Because decontamination with activated charcoal, fluid therapy and symptomatic treatment was performed for all patients, mean serum concentration of VPA, 2-ene, 4-ene were significantly reduced after 12 h.

One case received hemodialysis because the clinical condition and decrease in level of consciousness (Glasgow coma scale, 3). Life-threatening condition of this case wasn't firmly related to VPA concentration in plasma. The mean hospital stay for Spiller *et al*. (2000) patients was 42±33.1 h and in Isbister *et al*. (2003) study Most of the patients were discharged in under 24 h. The average duration of hospitalization was longer in Loghman poisoning ward (88.3 h) and all patients had normal blood tests, when they discharged. No case of death was observed.

It is notable that, all of over dosed cases were young and aged between 17-30 years old and 68% of them were female. None of them had accidental poisoning, so it is necessary psychiatric consultation to be done in all of the poisoned patients with this drug.

CONCLUSIONS

In this study, the level of VPA metabolites was less than toxic ranges and no case with hepatotoxicity, hyperammonia or cerebral edema was observed, but evaluation of serum levels of VPA metabolite could provide additional information about early detection of potential risk of hepatotoxicity and early treatment of side effects related to overdosing or idiosyncratic reactions.

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REFERENCES

Bojic, U., M.M. Elmazar, R.S. Hauck and H.Nau, 1996. Further branching of valproate- related carboxylic acids reduces the teratogenic activity, but not the anticonvulsant effect. Chem. Res. Toxicol., 9: 866-870.

Citrome, L., J. Levine and B. Allingham, 1998. Utilization of valproate: extent of in patient use in New York State Office of Mental Health. Psychiatr Q., 69: 283-300.

Cotariu, D. and J.L. Zaidman, 1988. Valproic acid and the liver. Clin. Chem., 34: 890-897.

Davis, R., D.H. Peters and D. McTavish, 1994. VPA A reappraisal of its pharmacological properties and clinical efficacy in epilepsy. Drugs, 47: 332-372.

Dupuis, R.E., S.N. Lichtman and G.M. Pollack, 1990. AcuteVPA overdose. Clinical course and pharmacokinetic disposition of VPA and metabolites. Drug Safety, 5: 65-71.

Fowler, G.W., 1978. Effect of dipropylacetate on serum levels on anticonvulsants in children. Proc. West. Pharmacol. Soc., 21: 37-40.

Franssen, E.J., G.G. van Essen, A.T. Portman, J. de Jong, G.Go, C.A. Stegeman and D.R. Uges, 1999. VPA toxicokinetics: Serial hemodialysis and hemoperfusion. Ther. Drug. Monit., 21: 289-292.

Garnie, R., O. Boudignat and P.E. Fournier, 1982. Valproate poisoning. Lancet, 2: 97-97.

Graudins, A. and C.K. Aaron, 1996. Delayed peak serum VPA in massive divalproex overdose-treatment with charcoal hemoperfusion. J. Toxicol. Clin. Toxicol., 34: 335-341.

Isbister, G.K., C.R. Balit, I.M. Whyte and A. Dawson, 2003. Valproate overdose: A comparative cohort study of self poisonings. Br. J. Clin. Pharmacol., 55: 398-404.

Kane, S.L., M. Constantiner, A.E. Staubus, C.D. Meinecke and J.R. Sedor, 2000. High-flux hemodialysis without hemoperfusion is effective in acute VPA overdose. Ann. Pharmacother., 34: 1146-1151.

Klotz, U. and K.H. Antonin, 1997. Pharmacokinetics and bioavailability of Sodium Valproate. Clin. Pharmacol. Ther., 21: 736-743.

Kondo, T., S. Kaneko, K. Otani, M. Ishida and T. Hirano *et al*., 1992. Associations between risk factors for valproate hepatotoxicity and altered valproate metabolism. Epilepsia, 33: 172-177.

Levy, R.H., B.S. Meldrum, R.H. Mattson and E. Perucca, 2002. Antiepileptic Drugs. 5th Edn., Lippincott Williams and Wilkins, New York, pp: 788.

Loscher, W. and H. Nau, 1982. VPA: metabolite concentrations in plasma and brain, anticonvulsant activity and effects on GABA metabolism during subacute treatment in mice. Arch. Int. Pharmacodyn Ther., 257: 20-31.

Meek, M.F., J. Broekroelofs, J.P. Yska, P.H. Egbers, E.C. Boerma and P.H. van der Voort, 2004. VPA intoxication: sense and non-sense of haemodialysis. Neth. J. Med., 62: 333-336.

Perucca, E., 2002. Pharmacological and therapeutic properties of valproate: A summary after 35 years of clinical experience. CNS Drugs, 16: 695-714.

Rettenmeier, A.W., W.P. Gordon, H. Barnes and T.A. Baillie, 1987. Studies on the metabolic fate of VPA in the rat using stable isotope techniques. Xenobiotica, 17: 1147-1157.

Riva, R., F. Albani, M. Contin and A. Baruzzi, 1996. Pharmacokinetic interactions between antiepileptic drugs. Clinical considerations. Clin. Pharmacokinet, 31: 470-493.

Robinson, P. and C. Abbott, 2005. Severe hypothermia in association with sodium valproate overdose. N. Z. Med. J., 118: U1681-U1681.

Schafer, H., 1990. Process for preparation (E)-2-propyl-2-pentenoic acid and physiologically compatible salts thereof. United States Patent 4945180. http://www.freepatentsonline.com/4945180.html.

Seger, D.L., 1998. Anticonvulsants. In: Clinical Management of Poisoning and Drug Overdose, Haddad, L.M., M.W. Shannon and J.F. Winchester (Eds.). 3rd Edn., WB Saunders, Philadelphi, pp: 655-63.

Spiller, H.A., E.P. Krenzelok and W..Klein-Schwartz, 2000. Multicenter case series of VPA ingestion: serum concentrations and toxicity. J. Toxicol. Clin. Toxicol., 38: 755-760.

Suchy, F.J., W.F. Balistreri, J.J. Buchino, J.M. Sondheimer and S.R. Bates *et al*., 1979. Acute hepatic failure associated with the use of sodium valproate. N. Engl. J. Med., 300: 962-966.

Sztajnkrycer, M.D., 2002. VPA toxicity: overview and management. Clin. Toxicol., 40: 789-801.

Tohen, M., J. Castillo, R.J. Baldessarini, C.Jr. Zarate and J.C. Kando, 1995. Blood dyscrasias with carbamazepine and valproate: a pharmaco epidemiological study of 2,228 patients at risk. Am. J.Sychiatry, 152: 413-418.

Ware, S. and C.H. Milward-Sadler, 1980. Acute liver disease associated with sodium valproate. Lancet, 2: 1110-1113.

Wilimowska, J., E. Florek and W. Piekoszewski, 2006. Disposition of VPA in self-poisoned adults. Basic Clin. Harmacol. Oxicol., 99: 22-26.