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A validated stability-indicating gas chromatography method for determination of divalproex sodium impurities in pharmaceutical preparation

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A stability-indicating gas chromatography (GC) method has been developed and validated for the quantitative determination of divalproex sodium impurities in pharmaceutical preparation. A technique has been developed whereby the peak purity of a compound with poor UV detection can be determined using a gas chromatograph coupled with a mass spectrometer. The drug products were subjected to hydrolysis, oxidation, photolysis, and heat to apply stress conditions. The stability-indicating nature of the method has been proven by establishing peak purity of all stressed samples. The chromatographic separation was performed on a fused silica capillary (Quadrex-FFAP, 30 meter, 0.32 mm and 1 μm film thickness) column. The method validation results indicate that the method is specific, accurate, linear, reproducible, rugged, and robust. The effectiveness of the technique was demonstrated with stability sample analysis of divalproex sodium in its pharmaceutical preparation.

Keywords: divalproex sodium; impurities; gas chromatography; stability-indicating method.

Introduction

Divalproex sodium is a stable co-ordination compound comprising sodium valproate and valproic acid (VPA) in a 1:1 molar ratio, as shown in Figure 1 and formed during the partial neutralization of VPA with 0.5 molar equivalent of sodium hydroxide. Chemically it is designated as sodium hydrogen bis(2-propylpentanoate). VPA (2-propylpentanoic acid) is a C8 branched carboxylic acid and an anti-epileptic drug widely used for the treatment of seizure disorder.[1] Many analytical approaches have been published for the determination of VPA, based on high performance liquid chromatography (HPLC),[2–8] capillary electrophoresis[9–10] in combination with mass spectrometry (MS),[11–17] ultraviolet detection (UV) or fluorescence detection, usually after dervatization with a suitable chromophore or fluorophore.[18–20] VPA and its pharmaceutical formulation is now official in USP forum and European Pharmacopoeia[21–22] but there no official or analytical method has appeared in the literature for impurity profiling of VPA in pharmaceutical formulations.

The pharmaceutical industry follows ethical rules and is bound to monitor strict control over the impurities when manufacturing drug substances and drug products. These impurities are classified as organic, inorganic and residual solvents.[23–24] Organic impurities can originate by alteration of reaction conditions, such as temperature, pH or in storage conditions (hydrolysis, oxidation, etc.). An ideal stability-indicating method is one that quantifies the standard drug alone and also resolves its degradation products. With the advent of International Conference on Harmonisation (ICH) guidelines, the requirement of establishment of stability-indicating method has become more clearly mandated. The guidelines explicitly require the conduction of forced decomposition studies under a variety of conditions, like pH, light, oxidation, dry heat, etc., and separation of the drug from degradation products. The establishment of purity of chromatographic peak in stressed samples is essential for the validation of the chromatographic method. This is particularly important when developing a stability-indicating method for determination of impurities. Currently a number of methods are employed for peak purity using a photo diode array detector, which essentially compares the entire UV spectra recorded at various points across the liquid chromatography (LC) peak with the spectrum collected at the apex of the peak. Sometimes this concept is not useful as there are often no spectral data available for the impurity.

Gas chromatography (GC) is a very well established technique for determination of residual solvents, drug testing and environmental contaminant identification. GC is very rarely used for quantitative determination of impurities in pharmaceutical preparation. Developing a stability-indicating method on GC for impurity profiling in pharmaceutical formulation is a challenging task. Complexities involved in development of method are extraction of impurities in presence of polymeric materials, selection of

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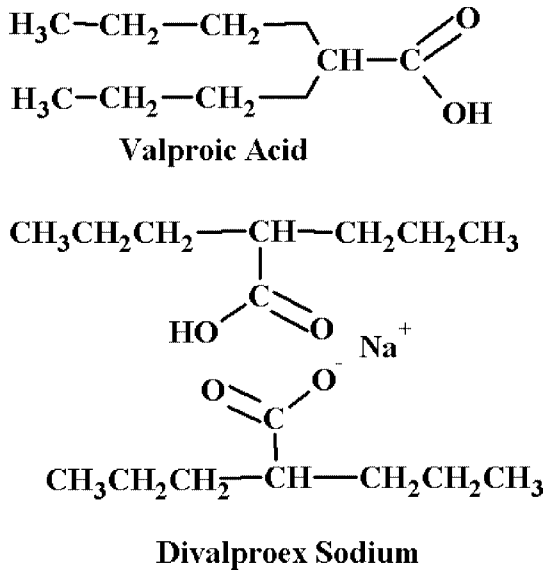

Figure 1. Chemical structures of Valproic Acid and Divalproex Sodium.

a suitable diluent for gas chromatographic analysis, and proving stability indicating nature of the method.

Several derivatization techniques were developed, in which VPA was converted into methyl ester derivatives, trimethylsilyl derivative, tetra-butyldimethylsilyl derivatives or pentafluorobenzyl derivatives.[25] Satisfactory results in terms of sensitivity have been obtained using GC-MS. However, all these methods are intended for determination of VPA in biological matrices, requiring multiple sample preparation, derivatization and are time consuming. In our study, a simple stability-indicating GC method was developed and validated for determination of related substance of divalproex sodium in pharmaceutical formulation. The peak purity of stressed samples has been established by comparing mass ion fragmentation pattern with the VPA reference standard. All four impurities (valeric acid, diethyl acetic acid, ethyl propyl acetic acid and diallyl acetic acid content) are well separated from each other. The method was validated as per ICH guideline[26] and successfully applied for separation of all compound of interest in the pharmaceutical formulation.

Experimental

Chemicals and reagents

VPA impurities were obtained from Dr Reddy's Laboratories, Hyderabd, India. Acetonitrile (HPLC grade), sulfuric acid (analytical reagent grade), chloroform (HPLC grade), formic acid, sodium sulfate, sodium hydroxide, hydrochloric acid, hydrogen peroxide were from Merck (Darmstadt, Germany). Water was purified by a Millipore (Bedford, MA, USA) Milli-Q water-purification system and passed through a 0.22 µm membrane filter (Durapore; Millipore, Dublin, Ireland) before use. Stock standard solutions of VPA and impurities were prepared in Milli-Q water as diluent.

Instrumentation

GC was carried out in an Agilent GC-FID consisting of 6890A GC and 5888N auto sampler. Quadrex-FFAP, 30 meter, 0.32 mm and 1 µm film thickness column was used. The injection volume was 4 µL. Oven temperature gradient was started at 60 °C held for 2 min, then raised to 230 °C at the rate of 3.5 °C/min and held at 230 °C for 20 min. Helium was used as carrier gas with a constant flow rate of 2 ml/min. The injector temperature was kept at 180 °C in split less mode. The detector temperature was kept at 240 °C. The specificity study was conducted by using heating oven, stability chamber and heating mantel (Thermo Lab Thane, India).

Standard and sample preparation

The standard stock solution of VAP was prepared by dissolving an accurately weighed amount of drug in diluent, resulting in a concentration of 1 mg/mL. Final standard solution was prepared by taking 5 mL of above stock solution, mixed with 21 mL of diluent and extracted three times with 20 mL chloroform each. Chloroform layer collected in a 100 ml flask after passing through sodium sulfate bed and volume made up to 100 mL.

The test solution was prepared by taking powdered tablets/capsules and a known amount of VPA equivalent to 4500 mg was transferred to a 50 mL volumetric flask along with 30 mL diluent. The powdered material was dispersed by mixing in an ultrasonic bath for 20 min and diluted to 50 mL with diluent. Above solution was centrifuged at 4000 rpm for 15 min in order to eliminate insoluble excipients and 20 mL of the supernatant liquid was taken for extraction. Test solution was mixed with 6 mL of 20% sulfuric acid solution to convert sodium valproate present in divalproex sodium into VPA and extracted three times with 20 mL chloroform each time. Chloroform layer was collected in a 100 ml flask after passing through sodium sulfate bed and volume made up to 100 mL. Standard and test preparation was used for chromatographic analysis.

Validation of the method

The method was validated for specificity, sensitivity, linear range, accuracy, precision and robustness as per ICH guidelines.[26]

Specificity

A study was conducted to demonstrate the effective separation of VPA and its impurities. The study was also intended to ensure the effective separation of degradation peaks of formulation ingredients at the retention time of VPA and its impurities. Separate portions of drug product and ingredients were exposed to following stress conditions to induce degradation.

The drug product was subjected to hydrolysis by refluxing the test solution in 5 N sodium hydroxide solution at 60 °C for 28 h. Similarly the acidic hydrolysis was performed by refluxing test solution in 5N hydrochloric acid solution at 60 °C for 28 h. The neutral hydrolysis was done in water at refluxing temperature of 60 °C for 24 h. Oxidation studies were performed in 3% hydrogen peroxide solution at 60 °C for 28 h. On photo stability study, the drug product was sufficiently spread on Petri-plates (1 mm thick layer) and exposed to sunlight and UV light at ambient conditions for 7 days. Humidity study was performed separately by exposing drug product to humidity at 25 °C, 90% RH for 7 days. Thermal degradation study was performed by heating drug product at 60 °C for 24 h. Similarly, placebo samples were prepared like a drug product by exposing formulation ingredients without drug substance. Stressed samples were injected into the GC system with FID detector by following test method conditions and same samples also analyzed in a GC coupled with mass detector to demonstrate peak purity.

Precision

The precision of test method was evaluated by analyzing six samples of VPA test preparation spiked with VPA impurities blend solution to get the concentration of 0.1% of sample concentration and analyzed as per test method.

Accuracy

A study of accuracy of VPA impurities from spiked samples of test preparation was conducted. Samples were prepared in triplicate by spiking impurities in test preparation at the level of limit of quantification (LOQ) 50%, 100%, 150%, 200% and 300% to the target concentration of impurities (i.e., About 0.2–80 µg mL^{-1}).

Limit of detection (LOD) and LOQ

LOD and LOQ values were determined by using the signal-to noise approach as defined in ICH guideline.[26] Increasingly, dilute solution of each impurity was injected and signal to noise was calculated at each concentration. LOD and LOQ values were calculated with a signal to noise ratio (S/N) 3 and 10 respectively.

Linearity of detector response

A series of solutions of VPA-related compounds in the concentration ranging from about LOQ level to about 300% (0.2–80 µg mL^{-1}) of the target concentration of impurities were prepared and injected into the GC system.

Real time sample analysis

The method suitability was verified by analyzing both initial and stability sample of in-house formulated product containing divalproex sodium as active substance. An accurately weighed quantity equivalent to 4500 mg of drug was taken and dispersed in 30 mL of diluent. The dispersed material was kept in an ultrasonic bath for 20 min and volume made up to 50 mL. Above solution was centrifuged at 4000 rpm for 15 min in order to eliminate insoluble excipients and 20 mL of the supernatant liquid was used for extraction. Test solution was mixed with 6 mL of 20% sulfuric acid solution and extracted thrice, each time with 20 mL chloroform. Chloroform layer was collected in a 100-ml flask after passing through sodium sulfate bed and volume was made up to 100 mL. Standard and test preparation were used for chromatographic analysis.

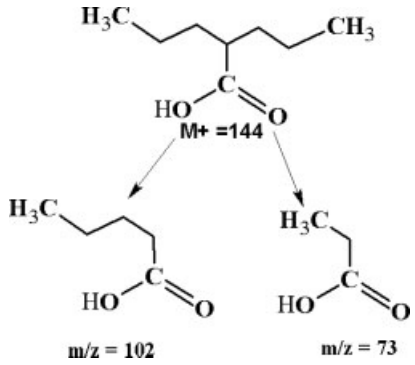

Figure 3. Chemical structures of Valproic Acid and its fragments.

Results and Discussion

In order to obtain the optimized extraction conditions and best extraction efficiency, we used the peak area of VPA standard as the GC response to evaluate the extraction efficiency under different conditions. To optimize the method, all extractions were initially carried out on standard stock solution and finally to tablets.

Divalproex sodium is a mixture of VPA and sodium valproate. Sodium valproate present in divalproex sodium was converted to VPA as sodium valproate is retained in column so thoroughly that it is not observed even after 75 min of injection, where as retention time and peak response of VPA are found to be satisfactory. VPA, with a pKa of 5, exists in neutral (un-ionized) form at low pH, and is completely ionized at pH higher than 4 and thus it has more tendency to dissolve in water. Divalproex sodium was dissolved in water, which was converted to VPA and sodium valproate. Subsequently, addition of acidic solution converted sodium valproate to VPA, as shown in Figure 2. The best results were obtained by adding 6 mL 20% v/v sulfuric acid solution.

It is essential to select a suitable organic solvent for GC analysis. The following factors should be considered. First, according to the theory of 'like attracts like', the extraction organic solvent should have high affinity for the analytes in the sample. Secondly, the organic solvent should be suitable for the GC analysis and the solvent peak should be satisfactorily resolved from the analyte peak. Finally, complete recovery in the presence of other excipient present in drug product is necessary.

Four solvents, N-Heptane, methanol, n-Hexane and chloroform were tested to select the best one for extraction of VPA in water samples with this technique. Preliminary experiments showed

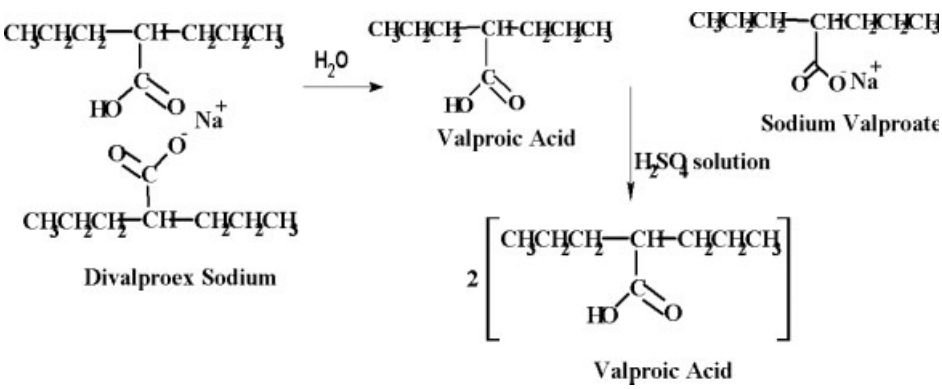

Figure 2. Chemical reaction of Divalproex Sodium to Valproic acid.

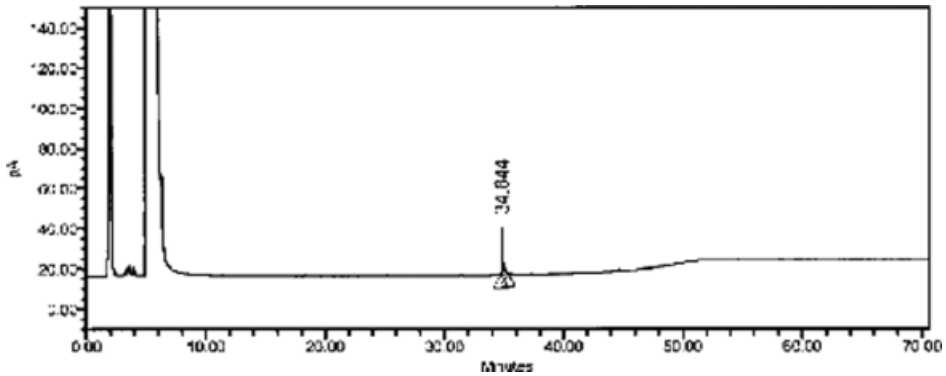

Figure 4A. Typical Chromatogram of Valproic Acid Standard.

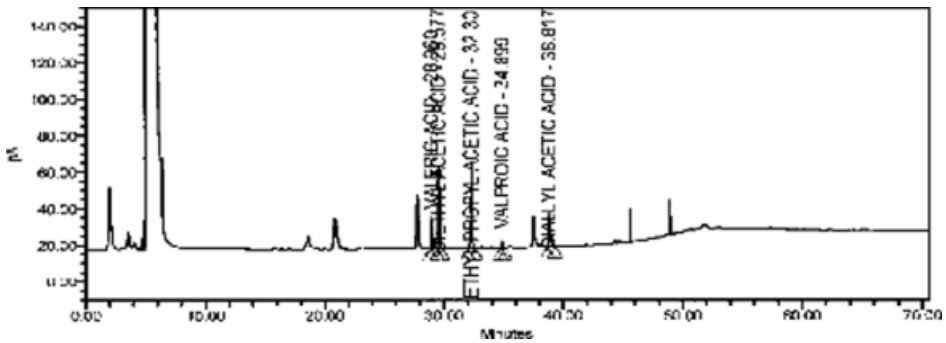

Figure 4B. Typical Chromatogram of Identification Solution.

that chloroform gives the best extraction efficiency of VPA in the presence of excipient matrix.

Selectivity, sensitivity, resolution, and speed of chromatographic separation were optimized for the GC method. The retention times of VPA at 35, valeric acid at 28.96, diethyl acetic acid at 29.58, ethyl propyl acetic acid at 32.30 and diallyl acetic acid at 38.82 min, respectively, under the chromatographic conditions described, and the total run time was 70 min. Chromatograms obtained from valproic acid standard, identification solution and test preparation are shown in Figures 4A, 4B and 4C, respectively.

The specificity of the proposed method was verified by injecting all stressed samples and placebo components. Further, all stressed samples were injected in to a GC coupled with mass detector to demonstrate the peak purity by following the standard test procedure. Upon evaluation, no ion was found overlapping with the main analyte peak in all the stressed samples. This indicates that there is no co-elution of degradation product peak with the main analyte peak. The main analyte peak in all the above samples was compared for the mass ion fragmentation pattern with the VPA reference standard. The observed possible fragmentation of main analyte peak is m/z = 73 and 102. Fragmentation patterns of all stressed samples and reference standards are the same. The structures of respective fragments are as given in Figure 3. The data also revealed that the known impurities along with the unknown degradation products are well resolved from the main peak.

Low relative standard deviation (RSD) values (<10%) of precision and intermediate studies indicate that the method is highly precise and data of precision study are shown in Table 1. The amount recovered was ±10% of amount added in accuracy study; this indicates that the method is highly accurate. The data of recovery study are shown in Table 2. Sensitivity of the method was verified

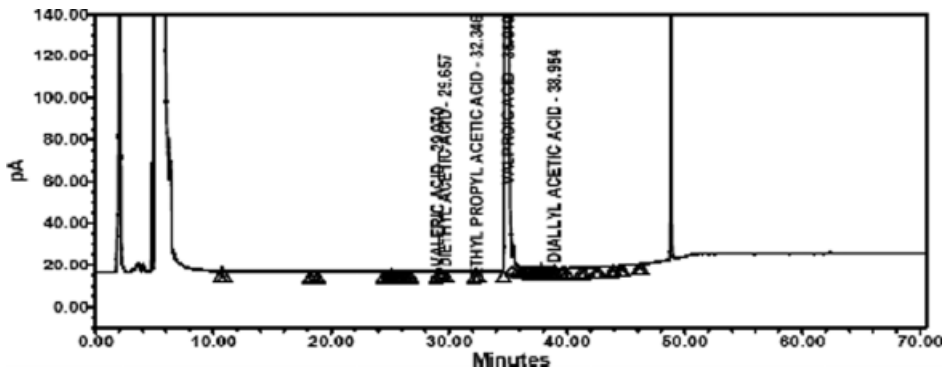

Figure 4C. Typical Chromatogram of Test solution.

Table 1. Percentage of RSD of impurities in precision study

Impurity Name	Precision (% RSD) ($n = 6$)	Intermediate Precision (% RSD) ($n = 6$)
Valeric Acid	1.8	2.6
Diethyl acetic acid	1.7	3.0
Ethyl propyl acetic acid	1.7	3.3
Diallyl acetic acid	2.0	3.4

Table 2. Percentage recovery of impurities at different level

Nominal concentrations	% Recovery of Valeric Acid	% Recovery of Diethyl Acetic Acid	% Recovery of Ethyl propyl Acetic Acid	% Recovery of Diallyl Acetic Acid
LOQ level	99.5	98.5	93.1	92.8
50% level	104.7	94.8	107.0	101.2
100% level	93.0	94.1	109.0	102.4
300% level	103.9	100.9	105.4	105.5

Table 3. Limit of detection (LOD) and limit of qualification (LOQ) of impurities in μg mL^{-1}

Nominal concentrations	Valeric Acid	Diethyl Acetic Acid	Ethyl Propyl Acetic Acid	Diallyl Acetic Acid
LOD μg mL^{-1}	0.1	0.1	0.05	0.1
LOQ μg mL^{-1}	0.5	0.4	0.4	0.5
Precission at LOQ (% RSD)	8.4	4.2	4.6	0.0

and the method was linear, accurate and precise at LOQ. The data of LOD and LOQ study are shown in Table 3. The calibration curve for all impurities was obtained by plotting the peak area of individual impurity versus the concentration over the range of about 0.2–80 μg mL^{-1}, and was found to be linear with $r = 0.999$. The data of regression analysis of the calibration curves are shown in Table 4. Real time analysis data ensured that the developed method is suitable for drug product analysis and data captured in Table 5.

An efficient, sensitive, selective, GC method has been developed and successfully applied in the separation of related substances of VPA. No interfering peaks were observed in blank and placebo, indicating that signal suppression or enhancement by the product matrix was negligible. A challenging task of peak purity has been established to prove that the method is stability indicating.

Conclusion

Although LC is a versatile technique for the impurity analysis in complex matrices, the presence of interfering substances and poor UV absorbency makes the separation and quantification difficult. A number of analytical approaches have been previously described to determine VPA in biological materials and pharmaceutical preparation; however, this is the first study reporting a validated stability-indicating GC method for impurity quantification in divalproex sodium formulation. The simple GC method developed in this study makes it suitable for separation and estimation of impurities without interference from excipients and other related substances present in the pharmaceutical matrices. Implementation of this GC method will help us to save organic solvent which will minimize environmental pollution. Development of a GC method is one of the best solutions to the current global acetonitrile shortage and will safeguard against future risk. The analytical performance and the result obtained from real time analysis demonstrate that the method is reliable. In conclusion, the sensitivity, selectivity, accuracy and reproducibility of the GC method developed in this study make it suitable for quality control analysis of complex pharmaceutical preparation containing divalproex sodium and its impurities.

Table 4. Correlation coefficient of impurities

Parameters (n = 7)	Valeric Acid	Diethyl Acetic Acid	Ethyl propyl Acetic Acid	Diallyl Acetic Acid
Slope	19.82	16.51	19.82	17.04
Y intercept	−21.1	−8.87	−21.10	59.89
Standard error	18.46	7.57	18.46	59.9
Correlation Coefficient	0.999	0.999	0.999	0.997

Table 5. Impurity profile of Depakote 500 mg delayed release tablets

	% impurity found	
Parameters	Initial	Stability (40°/75%RH, 3 Month)
Valeric Acid	BLD	BLD
Diethyl Acetic Acid	BLD	BLD
Ethyl Propyl Acetic Acid	BLD	BLD
Diallyl Acetic Acid	0.030	0.067
Single Max Unknown	0.009	0.018
Total Impurity	0.04	0.12

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