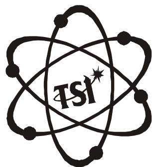

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A new validated liquid chromatographic method for the determination of impurities in fosphenytoin sodium

Sigala Ashok[1,2]*, M.Satish Varma[1] , CH.V.Raghunadha Babu[1], G.Balaswamy[2]

[1]Dr.Reddy's Laboratories Ltd., Integrated Product Development, Bachupally, Hyderabad-502325, (INDIA)

[2]Analytical Research, Integrated Product Development, Dr. Reddy's Laboratories Ltd., Kakatiya University, Warangal-506 009, (INDIA)

E-mail : sashok2k2@yahoo.com

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ABSTRACT

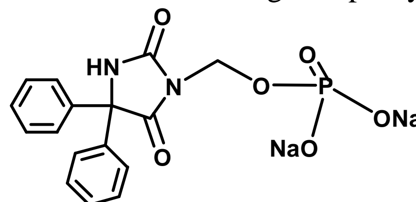
An improved gradient, reversed-phase liquid chromatographic (RP-LC) method has been developed and subsequently validated for the determination of Fosphenytoin sodium and its process-related impurities. Separation achieved with Symmetry shield RP-18, 250×4.6mm, 5µ column and a mobile phase of buffer (0.05 M monobasic potassium phosphate and 30mL of 0.5M dodecyltriethylammonium phosphate in 900ml of water, adjust with 1.5M phosphoric acid to a pH of about 3.0.) Acetonitrile with gradient elution at a flow rate of 1.0mL min^{-1}. UV detection was performed at 214nm. The described method is linear over a range of LOQ to 6.75µg mL^{-1} (150% of the specification limit) for all the process-related impurities. The accuracy of the method demonstrated and the recovery of impurities were found to be in the range of 85-110%. The method is simple, rapid, selective, accurate for the quantification of process related impurities of Fosphenytoin in its bulk drug samples. © 2010 Trade Science Inc. - INDIA

KEYWORDS

Fosphenytoin;
Process- related impurities;
RP-LC;
Validation.

INTRODUCTION

Fosphenytoin sodium is an anti-convulsant agent used for the treatment of epileptic disorder which is available in the market as Cerebyx[1,2]. Its chemical name is [2,5-dioxo-4, 4-di (phenyl) imidazolidin-1-yl] methyl dihydrogen phosphate sodium. It prevents the convulsion by modulating sodium channels of neurons. Fosphenytoin as a prodrug that provides phenytoin *in vivo* blocks movements of ions through the sodium channels during propagation of the action potential, and therefore blocks or limits the development of maximal convulsions. Fosphenytoin is a good example of a novel prodrug that can overcome the parenteral delivery problems associated with a sparingly watersoluble, weakly acidic drug. In reviewing the history and rationale behind this example it is hoped that the problems associated with the formulation of drugs like phenytoin could



2,4 Imidazolidinedione, 5,5 diphenyl-3-[(phosphonooxy) methyl],disodium

Fosphenytoin sodium

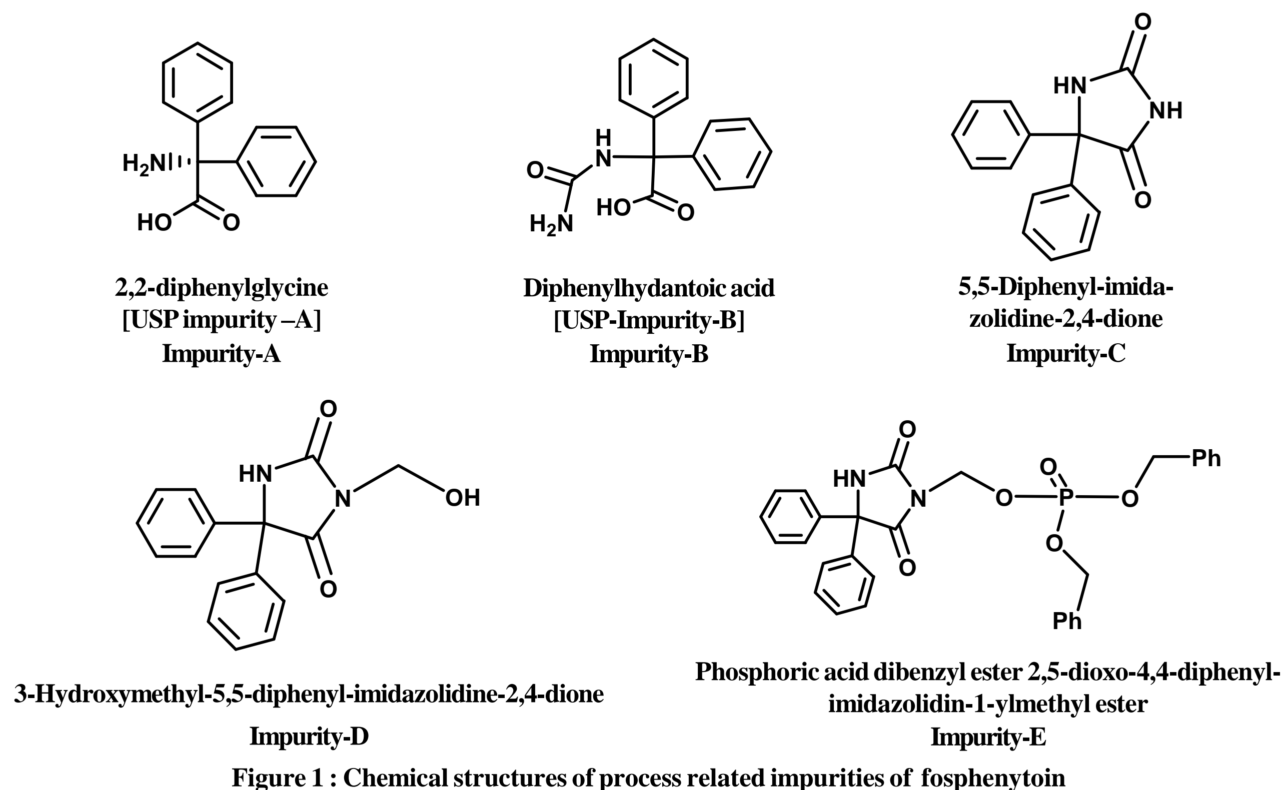


Figure 1 : Chemical structures of process related impurities of fosphenytoin

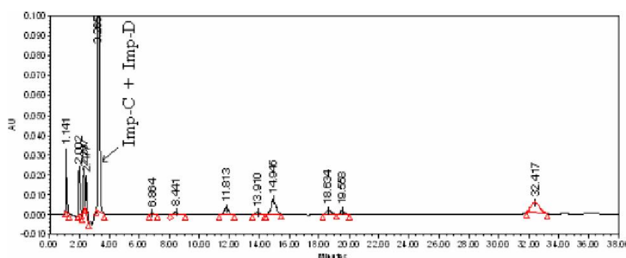


Figure 2 : Reference chromatogram as per USP

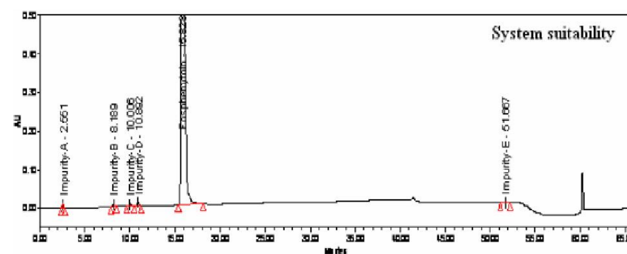


Figure 3 : Reference chromatogram of all impurities separation

be avoided in the future by prodrug intervention at the drug design stage. That is, if a drug such as phenytoin were to be discovered today, would it not be better to develop the parenteral form as fosphenytoin rather than sodium phenytoin, thus avoiding the safety and delivery limitations of the current product[3].

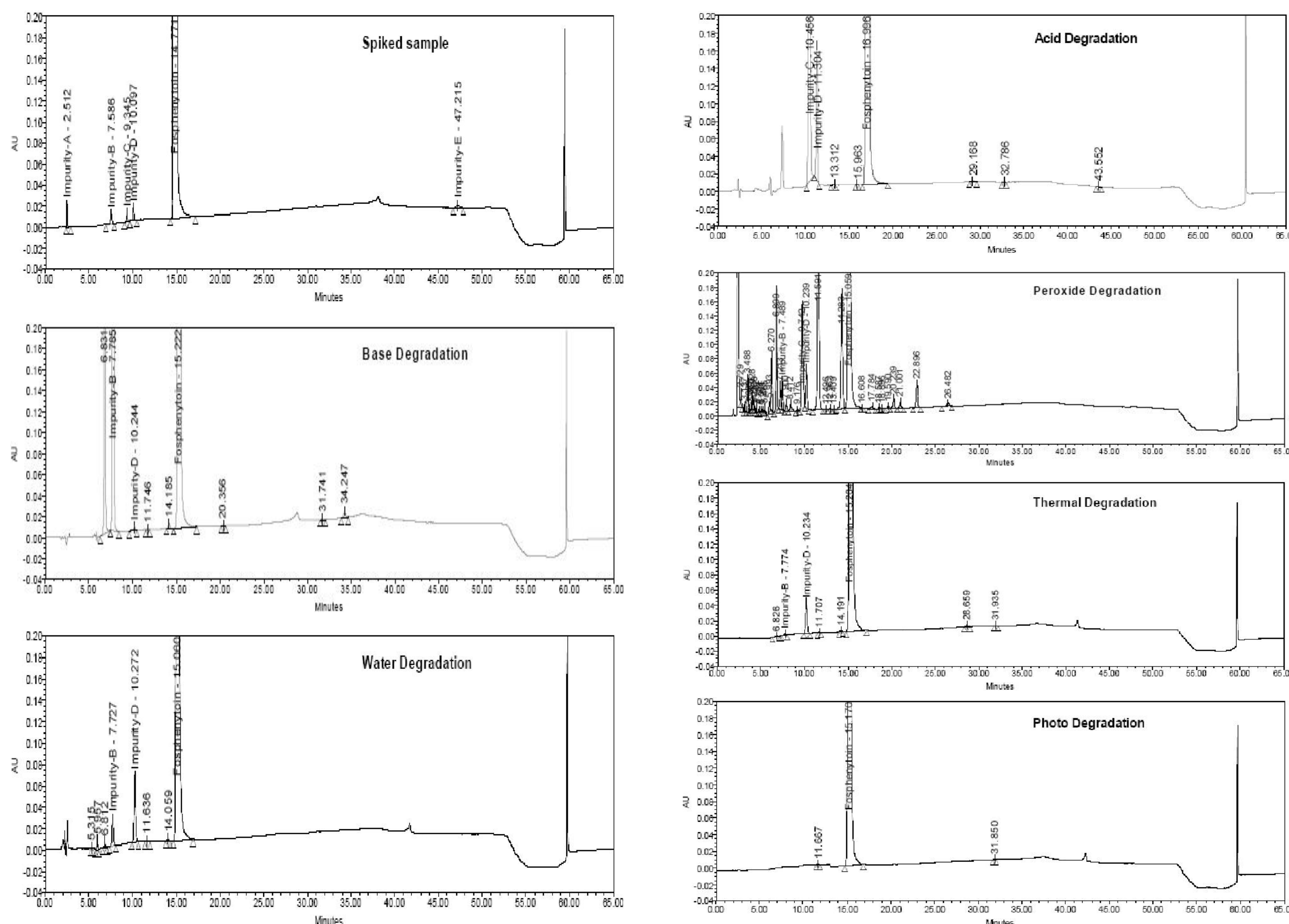
Fosphenytoin sodium is listed in official USP[4], to estimate its process related impurities. The USP specified method is not meeting the specificity criteria as described by ICH guidelines and also unable address the most probable impurities viz., Impurity –D and Impurity-E (Figure 1). The other literature reported method by Michael J.Cwik; et al.[5] is only suitable for the estimation of fosphenytoin and phenytoin in human plasma and plasma ultrafiltrate. Hence the author made an attempt to develop an appropriate method for the related substances determination.

Here, we present an improved analytical method for determination of process-related impurities in Fosphenytoin API, which will serve as a rapid and reliable method for the determination of process– related impurities in Fosphenytoin API. The method has been thoroughly validated as per the ICH guidelines[6].

EXPERIMENTAL

Chemicals

Samples of Fosphenytoin sodium and its related substances were received from process development laboratory of Dr.Reddy's Laboratories Ltd., IPDO, Hyderabad, India. The structures and chemical names of process-related impurities and Fosphenytoin sodium were shown in figure 1. HPLC grade Acetonitrile,



X-axis: Retention time in min and Y-axis: Peak response in mAU

Figure 4 : Typical chromatograms of fosphenytoin degradation samples and impurity spiked sample

monobasic potassium phosphate and ortho Phosphoric acid purchased from Merck, Germany. 0.5M dodecyltriethylammonium phosphate, purchased from Regis technologies Inc, USA and high pure water was prepared by using Millipore Milli Q plus purification system.

Instrumentation

The LC system, used for method development, forced degradation studies and method validation was Waters LC system with a diode array detector. The out put signal was monitored and processed using waters empower software on Pentium computer (Digital Equipment Co).

Chromatographic conditions

The chromatographic column used was a Symmetry shield RP-18, 250×4.6 mm, 5µ. The mobile phase –A contained buffer (0.05M monobasic potassium phosphate and 30mL of 0.5M dodecyltriethylammonium phosphate in 900 ml of water, adjust with 1.5 M phosphoric acid to a pH of about 3.0.) and Acetonitrile in the ratio of (75:25, *v/v*) and mobile phase- B consisted of Buffer and Acetonitrile in the ratio of (25:75, *v/v*). The flow rate of the mobile phase was 1.0mL min^{-1}. The LC gradient program was set as: time (min)/% mobile phase- B: 0.01/20, 2.5/20, 20/35, 35/50, 50/50, 52/20 and 65/20. The column temperature was maintained at 27°C and the detection was monitored at a wavelength of 214nm. The injection volume was 10µL. Buffer and Acetonitrile was used in the ratio of 65:35 as diluents for sample preparations.

Preparation of solutions

Preparation of spiked solution

All the process related impurities were spiked at a level of 4.5µg mL^{-1} to Fosphenytoin sodium standard solution, which is prepared at a concentration of 3.0mg mL^{-1} in diluent.

Sample solution

3.0mg mL^{-1} solution of Fosphenytoin sodium sample was prepared using diluent and injected into the system.

Forced degradation samples for specificity study

Fosphenytoin sodium was heated with aqueous 0.5N hydrochloric acid solution at 60°C for 18 hours and separately with aqueous 0.1N sodium hydroxide at 60°C for 1 hours to study formation of degradation products under acidic and basic conditions, respectively. Fosphenytoin sodium sample was heated with 3% hydrogen peroxide solution at 60°C for 24 hours to study formation of degradation products under oxidative condition. To study degradation products under photolytic and thermal degradations, Fosphenytoin sodium sample was exposed to ultraviolet light (254nm) for 10days and another sample was kept at 60°C temperature for 10 days respectively.

RESULTS AND DISCUSSION

Optimization of chromatographic condition

Fosphenytoin sodium is USP listed molecule. Impurities ABC (Figure 1) are the USP specified impurities. The monograph specified related substances by HPLC method is isocratic and also deficient to quantify the related impurities of fosphenytoin sodium. During the synthesis of the Fosphenytoin sodium there is a possibility of formation of impurity D and E (Figure 1) in addition to USP specified impurities. Initial trials were conducted exactly as per the USP specified chromatographic conditions. It is observed that the Impurity-C and impurity-D are eluting at the same retention time and impurity-E not eluting in USP chromatographic conditions (Figure 2). To overcome the drawbacks of the USP method further experiments were conducted. Further various trials were made by using different buffers like potassium phosphate, sodium phosphate, triethylamine and ammonium acetate in mobile phase to achieve desired separation between the impurity-C and impurity-D. Separation between the imp-C and imp-D did not improve even on varying the different buffers and column chemistries. Further experiments were conducted by varying the mobile phase pH as the two impurities were commonly containing the imidazolidine moiety. The pH of the USP buffer varied between 2 and 6. The separation is achieved between the two desired impurities when the mobile phase pH is maintained at pH 3.0 with a resolution about 1.4. Further to improve the resolution different stationery phases were checked. Achieved a resolution of greater 2.5 on symmetry shield RP 18 (2504.6mm, 5 micron) column. Further to elute the impurity-E introduced mobile phase-B with acetonitrile solvent under gradient conditions. It is also observed when the sample is injected as per the USP specified diluent, the product is undergoing degradation and forming the impurity-D. We could not differentiate this observation, upon analyzing the sample as per the USP specified chromatographic conditions. With the above experiments it is understood that the USP specified buffer pH 5.0 and the diluent are not appropriate for the related substances estimation of Fosphenytoin sodium.

Finally, derived the chromatographic conditions as buffer: 0.05M monobasic potassium phosphate mixed with 30mL of 0.5 M dodecyltriethylammonium phosphate in 900ml of water, adjusted to a pH of 3.0 with dilute phosphoric acid . The ratio of buffer and acetonitrile in (75:25, *v/v*) is used as mobilephase-A and the ratio of buffer and acetontirle in (25:75, *v/v*) is used as mobilephase-B. The gradient programme set as: time (min)/% mobilephase- B: 0.01/20, 2.5/20, 20/35, 35/50, 50/50, 52/20 and 65/20 with a flow rate of 1.0mL min^{-1} using symmetry shield RP-18 (250×4.6×5.0μ) column . The wavelength is monitored at 214nm. In these conditions all the related substances of Fosphenytoin are were eluted with good resolution (Figure 3).

Method validation

The LC method developed has been extensively validated for the process-related impurities of Fosphenytoin sodium using the following parameters.

Specificity

Specificity is the ability of the method to measure the analyte response in the presense of its potential impurities. Stress testing of a drug substance can help to identify the likely degradation products, which can in turn help establish the degradation pathways and the

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TABLE 1 : Linearity results for related substances estimation

Impurity Name	Slope	Y-intercept	Correlation coefficient
Impurity-A	1043.7	264.5	0.9995
Impurity-B	789.3	2419.5	0.9991
Impurity-C	825.2	1704.3	0.9991
Impurity-D	1108.3	4040.0	0.9992
Impurity-E	757.1	1590.0	0.9997

intrinsic stability of the molecule. The sample exposed to thermal and UV light not lead to any traceable degradation. But the sample heated with 0.5N HCl, 0.1N NaoH and 3% H_2O_2 were mostly converted to degraded products and these are well separated from the Fosphenytoin peak. Photodiode array detection was also used as evidence of the specificity of the method, and to evaluate the homogeneity of the peak. The sample exposed to acidic, basic, oxidative, thermal and UV light stress conditions were subjected to photodiode array analysis for peak purity of Fosphenytoin peak. The plots with flat tops in all instances showed that Fosphenytoin peak had no detectable impurity peaks embedded in and are free of co eluting degradation compounds. From the above results, it is clear that the method is specific and able to resolve all the process-related impurities and degradation products and can be used for determining the process related impurities in Fosphenytoin sodium API. A typical chromatogram of the degradation samples and spiked sample are shown in figure 4.

Linearity

Standard solutions at different concentration levels ranging from LOQ to 6.75μg mL^{-1} (150% of specification limit) were prepared and analyzed in duplicate in order to demonstrate the linearity for all the impurities. Linearity regression analysis demonstrated acceptability of the method for quantitative determination range of LOQ to 150% of specification limit. The coefficient of correlation was found to be more than 0.999. The values of slope, intercept and coefficient correlation for each impurity are shown in TABLE 1.

Accuracy

Accuracy of the method was demonstrated at four different concentration levels in triplicate. The analysis carried out at 50%, 75% 100% and 150% of specification limit. The mean recoveries of all the impurities were found to be in the range of 85-110% as shown in TABLE 2.

TABLE 2: Results of accuracy study

Impurity name	Spike level	Added (μg mL-1)	Recovered (μg mL-1)	(%) Recovery
Impurity-A	50	2.25	2.26	100.3
	75	3.38	3.44	101.7
	100	4.50	4.57	101.6
	150	6.75	6.79	100.7
Impurity-B	50	2.24	2.32	103.8
	75	3.38	3.46	102.4
	100	4.50	4.65	103.5
	150	6.75	6.70	99.3
Impurity-C	50	2.23	2.14	96.4
	75	3.38	2.97	87.9
	100	4.50	3.88	86.3
	150	6.75	6.10	90.4
Impurity-D	50	2.25	2.40	106.7
	75	3.38	3.65	108.2
	100	4.50	4.93	109.6
	150	6.75	6.89	102.2
Impurity-E	50	2.25	2.10	93.7
	75	3.39	3.21	94.9
	100	4.50	4.24	94.2
	150	6.75	6.47	95.9

Precision

Precision was determined through repeatability (intra-day) and intermediate (inter-day) precision. The precision of the related substance method was checked by injecting six individual preparations of (3.0mg mL^{-1}) Fosphenytoin sodium spiked with 0.15% of each impurity. The% RSD for percentage of each impurity was calculated. The intermediate precision of the method was evaluated by different analyst using different column and a different instrument in the same laboratory.

Limit of detection and limit of quantification

Sensitivity was determined by establishing the limit of detection (LOD) and limit of quantification (LOQ) for imp-A, imp-B , imp-C, imp-D and imp-E estimated at a signal to- noise ratio between 2-3 and 9.5-10.4 respectively, by injecting a series of dilute solutions with known concentration. The limit of detection of a com-

pound is defined as the lowest concentration that can be detected. LOD values were found to be 0.06, 0.12, 0.12,0.09 and 0.33μg mL^{-1} for imp-A, imp-B , imp-C , imp-D and imp-E, respectively. The limit of quantification is the lowest concentration of a compound that can be quantified with acceptable precision and accuracy. LOQ values were found to be 0.27, 0.45, 0.45, 0.36 and 1.23μg mL^{-1} for imp-A, imp-B , imp-C , imp-D and imp-E respectively. The precision study was also carried out at the LOQ level by injecting six individual preparations of imp-A, imp-B , imp-C , imp-D and imp-E and calculating the % RSD for the areas of each impurity.

Robustness

In order to demonstrate the robustness of the method, system suitability parameters were verified by making deliberate changes in the chromatographic conditions, viz, change in flow rate by ±0.2ml/min, change in pH of the buffer ±0.2 unit and changing the organic phase in the mobilephase from 100% to 90% and 110%. The method was demonstrated to be robust over an acceptable working range of its HPLC operational parameters.

CONCLUSION

The present paper describes the development of a new HPLC method for the determination of process-related impurities in Fosphenytoin sodium API and its validation. The method was found to be selective, sensitive, precise and accurate for the determination of process-related impurities and degradation products. This method can be used for the routine determinations in pharmaceutical quality control laboratories.

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