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Identification, Synthesis, Characterization and Quality Control Strategy of New Process-Related Impurities in Fosphenytoin Sodium

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Fosphenytoin sodium is a water dissolvable phenytoin prodrug that is directed intravenously to convey phenytoin, conceivably more securely than intravenous phenytoin. It is most ordinarily utilized in the intense treatment of convulsive status epileptics. The examination of the procedure-related contaminants will not help exclusively to advance the process parameters yet additionally to create sensible analytical methods and set the quality standard for a quality control system in pharmaceutical manufacturing. During the production of fosphenytoin sodium, all the process-related impurities are controlled in every stage and three degradation impurities are managed in the final API as per USP monograph. Besides, five unknown and one known contaminants were detected by HPLC method. All these impurities were identified, synthesized, isolated and characterized by IR, 1D-NMR (^{1}H, ^{13}C, DEPT) and HRMS spectral techniques. The mechanism of the formed impurities is examined for the first time. Quality control procedures to manage these impurities were developed to acquire the mass medication of ICH grade quality.

Keywords: Fosphenytoin sodium, Process related impurities.

INTRODUCTION

Status epileptics is a solitary epileptic seizure enduring over five minutes or at least two seizures inside five minutes without the individual coming back to ordinary between them [1]. Fosphenytoin sodium is broadly utilized in the treatment of status epileptics and has been affirmed worldwide under the brand name Cerebyx. The safety of a medication isn't just reliant on the toxicological properties of the mass medication itself; the contaminants in the mass medication also play a significant role. Consequently, the presence of these undesirable contaminations, even in limited quantities, may impact the viability and safety of pharmaceutical drugs. Therefore, the identification, measurement and control of impurities in the medication substance and the drug product are basic strides in tranquillize improvement and administrative appraisal.

The synthetic route of fosphenytoin sodium is shown in Fig. 1 [2-6]. The procedure-related impurities and degradation impurities of fosphenytoin sodium have also been shown in Fig. 1. In addition to the known impurities, five other unknown impurities are recognized on HPLC. All these obscure impurities in fosphenytoin sodium must be distinguished and portrayed by ICH rules [7-9]. As far as we could know, these obscure impurities have not been accounted for beforehand. When these mixes have been distinguished, it is critical to develop the synthetic and detection methods for them.

EXPERIMENTAL

Paraformaldehyde, potassium carbonate, other reagents and solvents were purchased from Loba Chemie (Mumbai, India) and sodium dibenzyl phosphate was purchased from Resin Allids (Gujarat, India). The HPLC grade acetonitrile (Merck), dodecyl trimethylammonium phosphate (Regis technologies Inc.), tetrabutylammonium bromide, phosphoric acid and potassium dihydrogen phosphate (KH_2PO_4) were purchased from Sigma-Aldrich. Water (Ultrapure) obtained from a Millipore water purification system (Bangalore, India) was used throughout the studies.

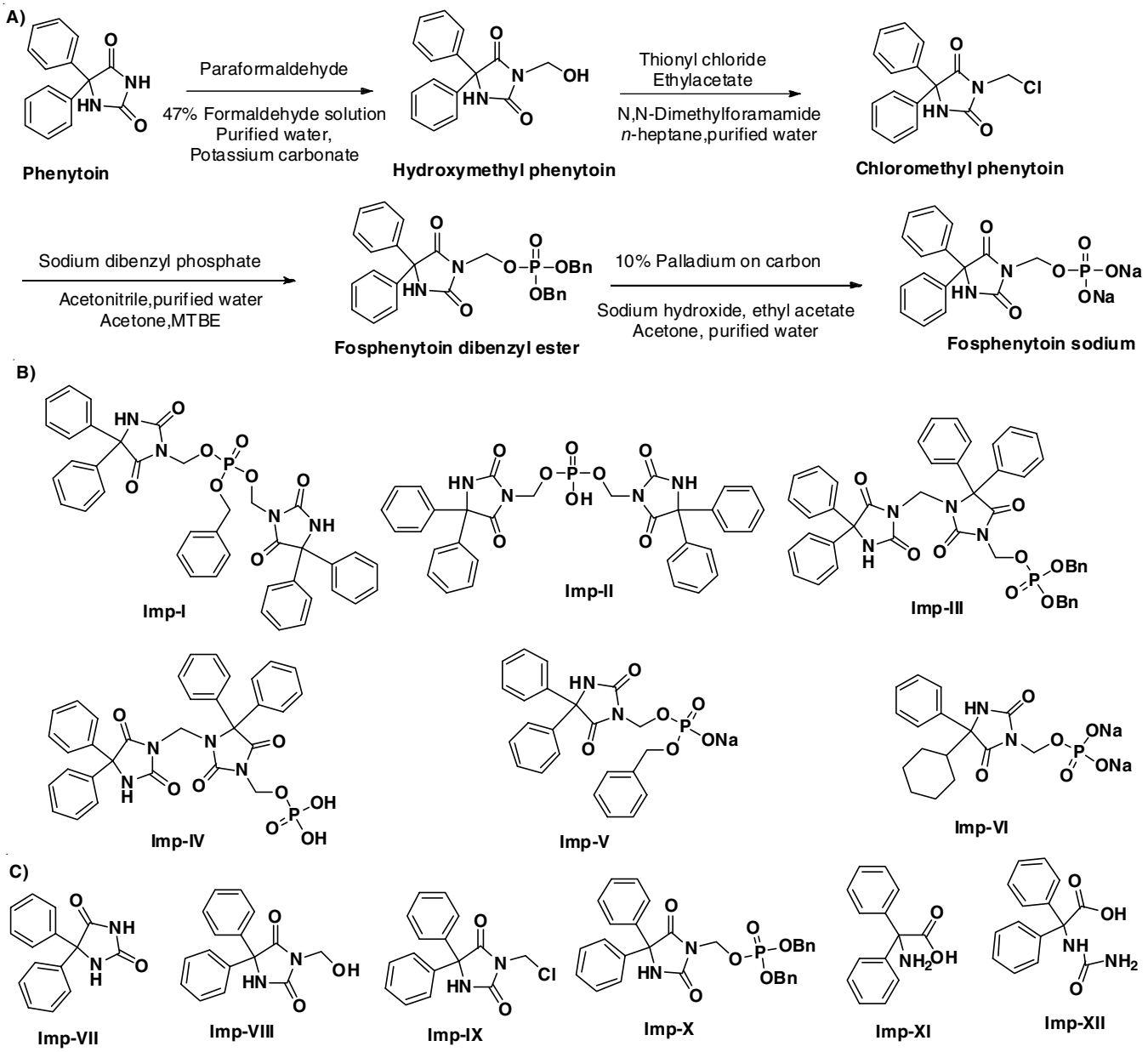


Fig. 1. (A) Route of synthesis of fosphenytoin sodium; (B) Structures of unknown impurities; (C) Structures of known impurities

HPLC conditions: The HPLC analyses were performed on a Shimadzu LC2010 HPLC instrument utilizing a reverse-phase symmetry C18, 150 mm × 3.9 mm, and 5 micron particle size column (Waters Corp, USA). Mobile phase A was a mixture of 35% ACN and 65% aqueous KH_2PO_4, 0.5M dodecyl trimethyl-ammonium phosphate, pH adjusted to 5.0 by using phosphoric acid and mobile phase B was a mixture of 50% ACN and 50% aqueous KH_2PO_4, 0.5M dodecyl trimethylammonium phosphate, pH adjusted to 5.0 by using phosphoric acid. The slope program was set as follows: (time (min)/% B: 0/0, 6/0, 16/100, 60/100, and 98/0). The column oven temperature was maintained at 25 ºC. The flow rate was kept at 1.0 mL/min and UV detection was recorded at 214 nm. The data acquisition time was 100 min.

NMR: Bruker Avance III instrument (Bruker, Karlsruhe, Germany) was used for recording the NMR spectra at 300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR with $(CH_3)_4Si$ (TMS) as an internal standard and are referenced using the central line of the solvent signal $CDCl_3$ (triplet at 77 ppm), DMSO-d_6 (septet at 39.5 ppm). DEPT experiments were also performed on a similar instrument to assign the correlations.

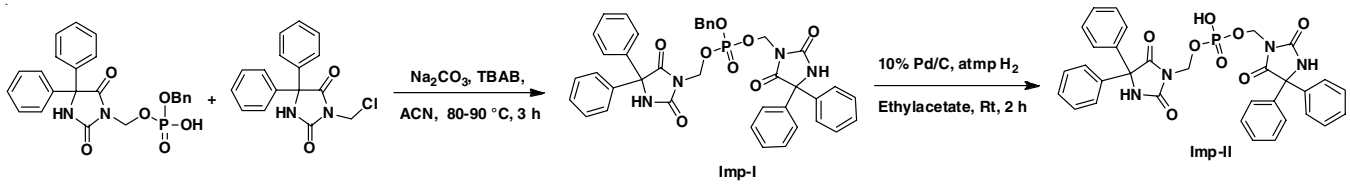
HRMS: HRMS spectra were recorded utilizing an instrument from Agilent QTOF 6530 model (Agilent Technologies Inc., CA, USA). The scan of fosphenytoin sodium and process related impurities in +ESI and –ESI mode were streamlined and the source parameters are as follows: Gas temp.: 200 ºC; Gas flow: 9 L/min; nebulizer: 40 psig; sheath gas temp.: 300 ºC; sheath gas flow: 9 L/min and scan portion positive polarity. The scan source parameters are VCap, 3200; nozzle voltage: 1000 V; fragmentor: 175; skimmer1, 65 and octopole RF peak: 750. The operating conditions of all the impurities and the drug were in +ESI and –ESI mode and recorded in the range of 110-1200 *m/z*.

Synthesis of Imp-I: To a stirred solution of benzyl-((2,5-dioxo-4,4-diphenylimidazolidine-1-yl)methyl)hydrogen phosphate (3.5 g, 7.73 mmol) in acetonitrile (175 mL) was added sodium carbonate (0.115 g, 1.08 mmol), tetrabutylammonium bromide (0.125 g, 0.38 mmol) followed by chloromethyl phenytoin (2.63 g, 8.75 mmol). The resulting reaction mixture was stirred at 80-90 ºC for about 2.5 h. The reaction mixture was concentrated under reduced pressure at 30-35 ºC to give a residue, which was purified by column chromatography using 100-200 mesh silica gel using a mixture of methanol (5%) and dichloromethane (95%) as eluent. The collected pure fractions were concentrated and the obtained product was dried under high vacuum at 30-35 ºC for 2 h to give a pure white colour solid (**Scheme-I**). Yield: 0.23 g, purity: 94%, ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 9.94 (s, 2H), 7.23-7.35 (m, 25H), 5.30-5.42 (m, 4H), 4.87 (d, 2H); ^{13}C NMR: 172.23, 153.17, 138.97, 128.55, 128.35, 128.29, 127.73, 126.65, 69.26, 68.99, 64.38.

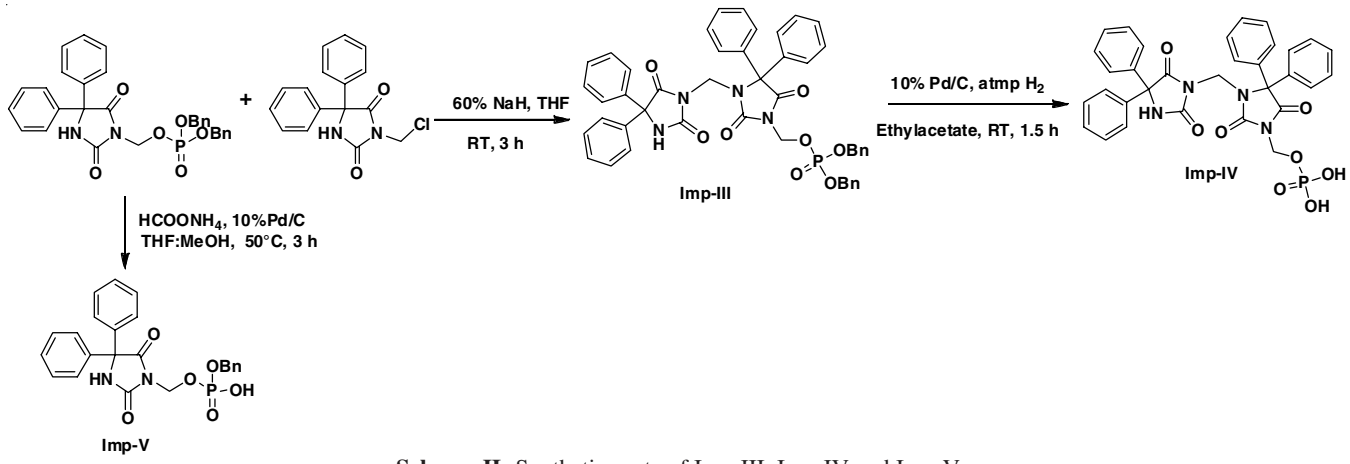
Synthesis of Imp-II: To a stirred solution of impurity I (350 mg) in ethyl acetate (3 mL) was added 10% Pd/C (0.035 g). The resulting reaction mixture was stirred under H_2 pressure at room temperature for about 1.5 h. After the completion of the reaction, which was monitored by TLC, the reaction mixture was filtered through hyflo-bed and washed with ethyl acetate. The obtained filtrate was concentrated under reduced pressure at 30-35 ºC for 2 h. The obtained crude compound was purified by column chromatography using 100-200 mesh silica gel using a mixture of methanol and dichloromethane as eluent. The collected pure fractions were concentrated and the obtained product was dried under high vacuum at 30-35 ºC for 2 h to give a pure white colour solid (**Scheme-I**). Yield: 0.22 g; purity: 88%, ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 9.85 (bs, 2H), 7.36 (s, 20H), 5.12 (bs, 4H); ^{13}C NMR: 172.62, 154.12, 139.38, 128.52, 128.18, 126.69, 68.99, 62.89.

Synthesis of Imp-III: Impurity X (5.0 g, 9.22 mmol) was taken in tetrahydrofuran (20 mL) and cooled to 0-5 ºC. Sodium hydride was then added portion-wise with stirring at the same temperature. After complete addition, the reaction mixture was stirred at 0-5 ºC for about 30 min. To this, impurity IX (3.0 g, 10.14 mmol) was added. The resulting reaction mixture was stirred at 25-30 ºC for about 3 h. The reaction was monitored by TLC. Upon completion of the reaction, the reaction mixture was concentrated under vacuum at 30 ºC. The obtained crude compound was purified by column chromatography using 100-200 mesh silica gel and the product was eluted at 3% methanol in dichloromethane. The collected pure fractions were concentrated and the product obtained was dried under high vacuum at 30-35 ºC for 2 h to give a pure white colour solid (**Scheme-II**). Yield: 0.3 g; purity: 94%, ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.06-7.33 (m, 30H), 5.49 (d, 2H) 5.16 (s, 2H), 4.94-5.04 (m, 4H); ^{13}C NMR: 172.15, 171.78, 154.95, 154.12, 138.62, 135.50, 134.80, 126.47-129.18, 74.93, 69.71, 64.49.

Synthesis of Imp-IV: To a stirred solution of impurity III (0.35 g, 0.43 mmol) taken in ethyl acetate (6 mL) was added 10% Pd/C (0.035 g). The resulting reaction mixture was stirred under H_2 bladder pressure at room temperature for about 1.5 h. The completion of the reaction was monitored by TLC and the reaction mixture was filtered through hyflo-bed and washed the bed with ethyl acetate. The filtrate was concentrated under reduced pressure at 30-35 ºC. The obtained crude compound was purified by column chromatography using 100-200 mesh silica gel and the product was eluted at 5% methanol in dichloromethane. The collected pure fractions were concentrated and dried under high vacuum at 30-35 ºC for 2 h to give a pure white colour solid (**Scheme-II**). Yield: 0.22 g; purity: 96%, ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.70 (bs, 1H,), 7.05-7.26 (m, 20H), 5.39 (s, 2H) 4.98 (s, 2H); ^{13}C NMR: 172.16, 155.65,



Scheme-I: Synthetic route of Imp-I and Imp-II



Scheme-II: Synthetic route of Imp-III, Imp-IV and Imp-V

154.86, 138.43, 134.54, 126.58-129.15, 74.89, 69.59, 64.5, 45.02.

Synthesis of Imp-V: To a stirred solution of FPS stage-III compound (15.0 g, 27.65 mmol) taken in a mixture of solvents THF: MeOH (225 mL) was added ammonium formate (91.74 g, 27.65 mmol) followed by 10% Pd/C (2.25 g). The resulting reaction mixture was stirred at 50 ºC for about 3 h. After the completion of the reaction which was monitored by TLC analysis, the reaction mixture was filtered through a bed of hyflo, washed with methanol and then concentrated under vacuum at 30 ºC. The obtained crude compound was purified by column chromatography using 100-200 mesh silica gel using a mixture of methanol (5%) and dichloromethane (95%) as an eluent. The pure fractions were concentrated and dried under high vacuum at 30-35 ºC for 2 h to give a pure white color solid compound (**Scheme-II**). Yield: 6.0 g; purity: 94.0%, ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 9.77 (bs, 1H), 7.28-7.36 (m, 15H), 5.07 (d, 2H) 4.63 (d, 2H); ^{13}CNMR: 172.73, 154.29, 139.46, 128.48, 128.14, 127.94, 127.09, 126.95, 126.65, 68.98, 65.97, 62.87.

Synthesis of Imp-VI: Fosphenytoin sodium-containing about 5% impurity VI was purified by flash column chromatography using C18 silica gel column with 0.1% TFA containing water and acetonitrile mixture as a gradient eluent. The pure fractions collected was concentrated, and the material obtained was dried under high vacuum at about 30 ºC for 2 h to give an off white colour solid compound. The compound obtained was dissolved in 1.5 mL of water; to that 1.5 equivalent of NaOH solution was added. The solution obtained was precipitated with acetone, filtered, washed twice with acetone and dried for 2 h at about 30 ºC to give a pure white colour solid compound (**Scheme-III**). Yield: 50 mg; purity: 95%, ^{1}H NMR (400 MHz, D_2O): δ ppm: 7.41-7.60 (m, 5H), 5.07 (d, 2H) 2.37-2.42 (m, 1H), 1.56-1.78 (m, 4H), 0.89-1.30 (m, 6H); ^{13}C NMR: 177.59, 157.78, 136.66, 128.92, 128.46, 125.78, 71.77, 62.85, 44.96, 26.47, 26.18, 25.5.

RESULTS AND DISCUSSION

Identification of impurities: A typical analytical HPLC chromatogram of fosphenytoin sodium along with their spiked impurities was recorded using the HPLC and shown in Fig. 2. The structures, relative retention time (RRT), molecular weight (m.w.) and nature of these impurities are given in Table-1.

Structural elucidation of synthesized impurities

Characterization of Imp-I: The ESI-MS spectrum of Imp-1 showed a molecular ion peak at *m/z* 717.21 ([M+H]$^+$) in positive ion mode, which complies with the Imp-I molecular weight is 716.67. In the ^{1}H NMR spectrum, aromatic protons are clubbed together and appeared peak at 7.23-7.35 ppm. The benzyl CH_2 appeared at 4.87 (2H, doublet). The two -NCH_2 groups have appeared at 5.30-5.42 (4H, multiplet). The peak appeared at 9.94 corresponds to two -NH protons (2H, singlet). The DEPT-135 NMR spectrum shows five peaks above the plane and two peaks below the plane confirming that the compound having 25CH groups (16CH groups are in two different sets of similar environment, 3CH groups are in one set of similar environment, 2CH groups are in one set of similar environment and 4CH groups are in one set of similar environment) and 3CH_2 groups (of which 2CH_2 groups are in similar environment) in the molecule.

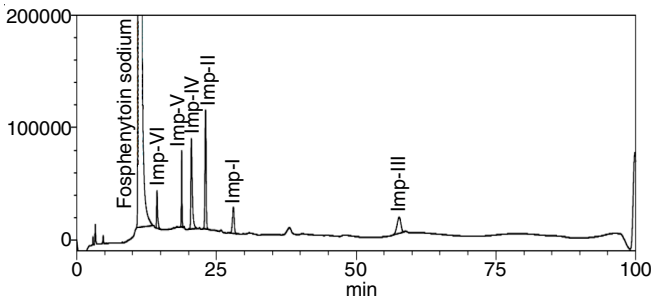
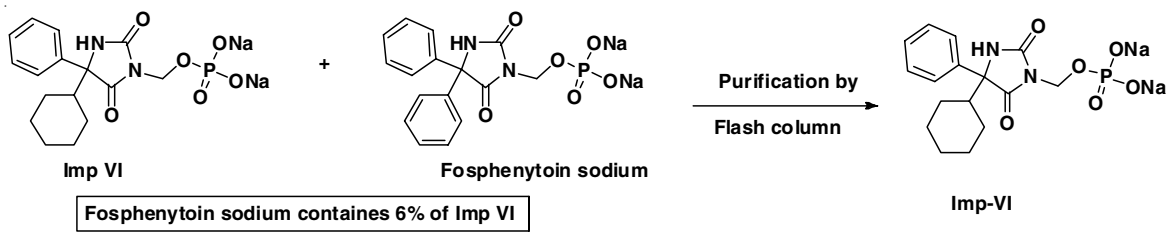


Fig. 2. HPLC chromatogram of Fosphenytoin sodium spiked with unknown impurities

TABLE-1
CRITICAL INFORMATION ABOUT THE IMPURITIES OF BULK FOSPHENYTOIN SODIUM

Compound	RRT	m.w.	Nature
Fosphenytoin sodium	1.0	406.24	Drug substance
Impurity I	2.52	716.67	Process related
Impurity II	2.08	626.56	Process related
Impurity III	5.20	806.81	Process related
Impurity IV	1.85	626.56	Process related
Impurity V (known impurity)	1.69	474.38	Process related
Impurity VI	1.29	369.12	Process related

Characterization of Imp-II: The ESI-MS spectrum of Imp-II shows a molecular ion peak at *m/z* 626.16 ([M+H]$^+$) in positive ion mode, indicating that the molecular weight is 627.16. Aromatic protons appeared at 7.36 (20H, singlet), two -NCH_2 protons have appeared at 5.12 (4H broad singlet), two -NH protons appeared at 9.85 (2H, broad singlet). The DEPT-135 NMR spectrum shows three peaks above the plane and one peak below the plane confirming that the compound is having 20CH groups (16CH groups are in two different sets of similar environment and 4CH groups are in one set of similar environment) and 2CH_2 groups (both are in the same environment) in the molecule.



Scheme-III: Synthetic route of Imp-VI

Characterization of Imp-III: The ESI-MS spectrum of Imp-III shows a molecular ion peak at *m/z* 805.24 ($[M-H]^-$) in negative ion mode, indicating that the molecular weight is 806.24. In ^1H NMR spectrum of impurity III, aromatic protons appeared to peak at 7.06-7.33 (30H, multiplet). The two benzyl CH_2 appeared at 4.94-5.04 (4H, multiplet). The -NCH_2 attached to phosphate groups appeared at 5.49 (2H, doublet). The -NCH_2 appeared at 5.16 (2H, singlet). The DEPT-135 NMR spectrum shows nine peaks above the plane and three peaks below the plane confirmed that the compound has 30CH groups that are in nine different sets of similar environment and 4CH_2 groups (of which 2CH_2 groups are in a similar environment) in the molecule.

Characterization of Imp-IV: The ESI-MS spectrum of Imp-IV shows a molecular ion peak at *m/z* 627.15 ($[M+H]^+$) in positive ion mode indicating that the molecular weight is 626.16. In ^1H NMR spectrum of impurity IV, all the aromatic protons merged and appeared at 7.05-7.26 (20H, multiplet). The appeared at 5.39 (2H, singlet) corresponds to -NCH_2 attached to the phosphate group. The peak appeared at 4.98 (2H, singlet) belongs to -NCH_2 protons. The DEPT-135 NMR spectrum shows eight peaks above the plane and two peaks below the plane confirming that the compound has 20 CH groups that are in eight different sets of similar environment and 2 CH_2 groups in the molecule.

Characterization of Imp-V: The ESI-MS spectrum of Imp-V (known impurity) shows a molecular ion peak at *m/z* 453.12 ($[M+H]^+$) in positive ion mode, indicating that the molecular weight is 452.11. In ^1H NMR spectrum of impurity V, the aromatic protons have appeared at δ 7.28-7.36 ppm (15H, multiplet). Benzyl -CH_2 protons appeared at δ 4.63 ppm (2H, doublet). The peak appeared at δ 5.07 ppm (2H, doublet) corresponds to -NCH_2 protons. The peak appeared at δ 9.77 ppm (1H, broad singlet) belongs to -NH proton. The DEPT-135 NMR spectrum shows six peaks above the plane and two peaks below the plane confirming that the compound is having 15CH groups (8CH groups are in two different sets of similar environment, 5CH groups are in three different sets of the same environment and the remaining 2CH groups are in similar environment) and 2CH_2 groups in the molecule.

Characterization of Imp-VI: The ESI-MS spectrum of Imp-VI shows a molecular ion peak at *m/z* 369.12 ($[M+H]^+$) in positive ion mode, indicating that the molecular weight is 368.11. In ^1H NMR spectrum of impurity VI, the aromatic protons appeared at δ 7.41-7.60 ppm (5H, multiplet). In cyclohexyl group, C10 proton appeared at δ 2.37-2.42 ppm, the peak appeared at δ 0.89-1.30 ppm (6H, multiplet) belongs to C-8H, C-8′H & C-9H protons, and the peak appeared at δ 1.56-1.78 ppm (4H, multiplet) belongs to C-7H, C-7′H. The peak appeared at δ 5.07 ppm (2H, doublet) corresponds to -NCH_2 protons. The DEPT-135 NMR spectrum shows four peaks above the plane and six peaks below the plane, confirming that the compound is having 6 CH groups and 6 CH_2 groups in the molecule.

Control of impurities: One of our primary objectives is to investigate how these process-related impurities controlled during synthesis or through a purification procedure to produce high-quality pharmaceuticals. If we realize the route cause for the formation of process-related impurities, it is easy to design a rational process to reduce or avoid the development of contaminations or by altering the workup procedure to remove the formed impurities indicated by the idea of quality by design (QBD). In present API process, six impurities in the final product, including the four known impurities are identified and controlled in the final API to be less than 0.10% by HPLC. All of these impurities are evacuated to accomplish ICH-grade quality.

Conclusion

Isolation and successful synthesis of these unknown process related impurities [Imp-I, Imp-II, Imp-III, Imp-IV, Imp-V (known impurity) and Imp-VI] were possible during the manufacturing of fosphenytoin sodium from phenytoin. All the impurities are observed to be less than 0.10% based on HPLC analysis. Six unknown impurities were identified, synthesized, isolated and characterized by using IR, NMR and HRMS techniques. The formation mechanisms of these obscure polluting influences are also discussed. Improved procedures of impurity control were created by the physical and chemical properties of these contaminations. At long last, because of the above studies, fosphenytoin sodium of ICH-grade quality was effectively acquired.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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