SOURCES OF IMPURITIES - INVESTIGATION OF 4-(2-CHLOROPHENYL)-3-ETHOXYCARBONYL-5-METHOXYCARBONYL-6-METHYL-2-[(2-PHTHALIMIDOETHOXY)METHYL]-1,4-DIHYDROPYRIDINE TRACES FORMATION DURING THE SYNTHESIS OF AMLODIPINE BESYLATE

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Abstract

General consideration of possible sources of impurities in organic synthesis products is presented, distinguishing between primary and secondary impurities. By an investigation of impurity **3** in amlodipine besylate **1** it is shown that packaging and process equipment materials, involved in a synthesis, can be additional source of impurities. It was found that impurity **3** can be formed by reaction between amlodipine besylate **1** and bis(2-ethylhexyl) phtalate (**2**) eluted from plastic materials used for storage of solvents for the amlodipine besylate synthesis. A selective and sensitive HPLC-MS/MS method was developed for this investigation.

Investigation of impurity profile of an active pharmaceutical ingredient is of crucial importance for medical safety reasons. The level of impurities must be reduced typically to less than 1%, and each individual impurity of 0.1% and above must be identified [1]. Therefore a lot of efforts during a development of synthetic technologies is focused to minimization of the impurities. To achieve this, identity and origin of each impurity is essential.

Theoretically, impurities of a synthetic product can be:

- *residues* of intermediates, by-products, starting materials and their impurities
- *degradation products* of a desired product, intermediates, by-products, starting materials and their impurities
- *products of reactions* among all above species.

Further elaboration of above theoretically possible (potential) impurities leads to the following two types of impurities:

- impurities from formation of a desired product, such as by-products, residues of starting materials and intermediates (primary impurities)
- impurities deriving from impurities of the mainstream components, i.e. residues of impurities of starting materials, degradants of impurities, products of reactions of and among impurities etc. (secondary impurities).

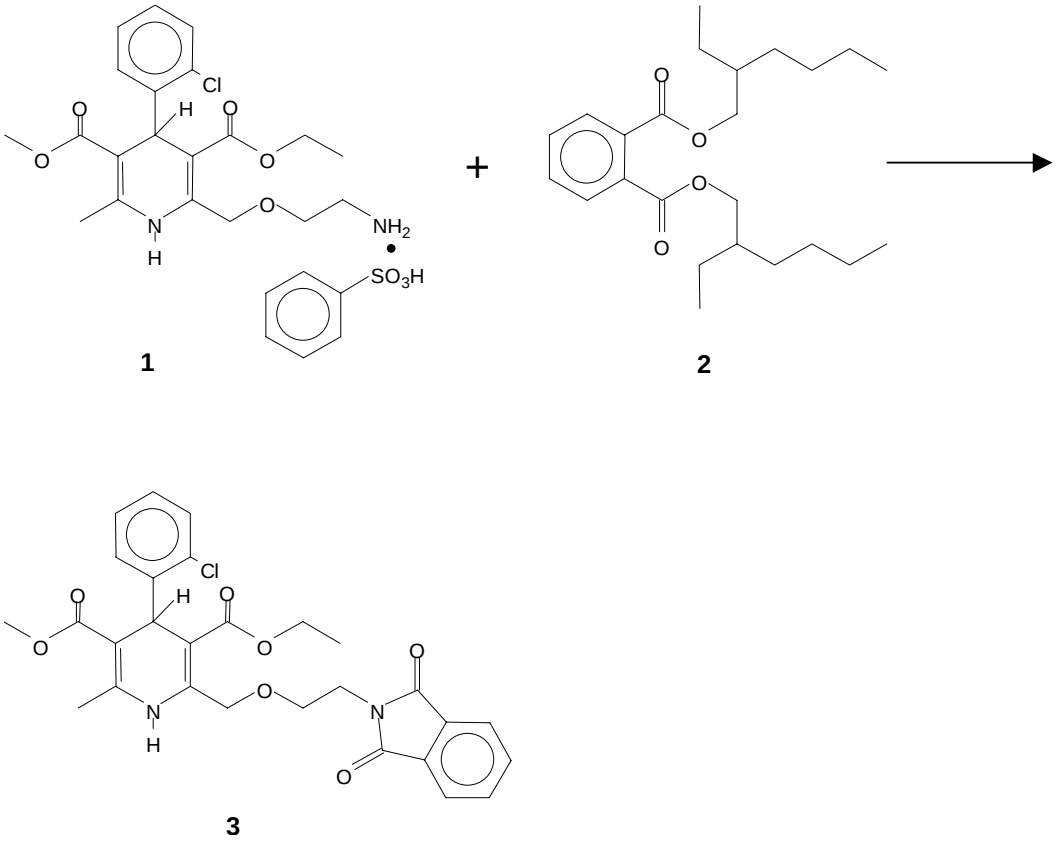
While primary impurities are typically formed at higher levels, and are therefore considered by regulatory authorities [1, 2], secondary impurities are usually formed at very low levels, such as ppm or ppb, and are more important for forensic reasons when looking for specific (characteristic) impurities for a particular synthetic route, often used in order to establish patent infringement. Reactions at the impurity (trace) level are less known and are not systematically studied.

In our case investigation of impurities at the ppb level in the amlodipine besylate, 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihidropyridine benzenesulphonate [3, 4], long acting dihydropyridine type Calcium channel blocker **1**, synthesized according to the Lek's synthetic procedure [5], revealed an additional possible source of impurities: components from packaging and process equipment materials that are involved in a synthetic process.

The synthesis of amlodipine besylate in consideration is based on Hantzch cyclization to substituted dihydropyridine ring with triphenylmethyl protected amino group - this protecting group is easily removed by excess of benzenesulphonic acid in an appropriate solvent to form amlodipine benzensulphonate [5]. Detailed analysis of impurities in product obtained showed that it contains traces of phthalimido derivative **3** in spite of the fact that no phthalimido derivatives were involved in the synthesis and that it was not treated as a theoretically possible (potential) impurity on the basis of the above possible sources. Therefore we were searching for additional sources to explain the presence of this impurity and found that it is formed at least by reaction of amlodipine besylate **1** with bis(2-ethylhexyl) phthalate (**2**) (Scheme I), which is released from plastic packaging and process equipment materials [7]. Since the reaction slowly occurs at elevated temperature

the impurity **3** is most obviously formed during hydrolysis and recrystallization, but additional mechanisms of its formation (e.g. reactions of **2** in earlier phases of the synthesis) are not excluded. Investigation of impurity **3** was performed with a selective and sensitive HPLC-MS/MS method.

Scheme 1. Formation of phthalimido derivative **3** from amlodipine benzenesulphonate **1**.



EXPERIMENTAL

Materials

Amlodipine benzensulphonate of Lek d.d. [5], methanol HPLC grade of Riedel-de-Haen, bis(2-ethylhexyl) phthalate of Merck Darmstadt were used for the reaction. Phthalimido intermediate **3**, used as an analytical standard, was prepared by Lek according to the published procedure [6], m.p. 148-149°C, lit. m.p. 146-147°C [6].

^{1}H-MNR chemical shifts (ppm) in $CDCl_3$: 7.89 (m, 2H, phthalimide r.), 7.77 (m, 2H, phthalimide r.), 7.35 (dd, J=7.6, 1.5 Hz, 1H, o-Cl-phenyl), 7.21 (dd, J=7.6, 1.5 Hz, 1H, o-Cl-phenyl), 7.09 (td, J=7.6, 1.5 Hz, 1H, o-Cl-phenyl), 7.02 (td, J=7.6, 1.5 Hz, 1H, o-Cl-phenyl), 5.37 (s, 1H, H-4), 4.73 and 4.64 (d, J=16.0 Hz, 1H, 2-$\underline{CH_2}OCH_2CH_2$- phthalimide r.), 4.02 (m, 4H, 2-$CH_2O\underline{CH_2}CH_2$- phthalimide r., 3-$COO\underline{CH_2}CH_3$), 3.77 (m, 2-$CH_2OCH_2\underline{CH_2}$- phthalimide r.), 3.61 (s, 3H, 5-$COO\underline{CH_3}$), 2.43 (s, 3H, 6-CH_3), 1.16 (t, J=7.3 Hz, 3H, 3-$COOCH_2\underline{CH_3}$)

Analyses were performed by HPLC-APCI-MS/MS method using Constametric 4100 MS pump (LDC Analytica, Riviera Beach, FL, USA), HPLC autosampler AS 3000 (TSP Separations, Riviera Beach, FL, USA) and mass spectrometer TSQ 7000 (Finnigan MAT, San Jose, TE, USA), on HPLC column Hicarbosphere UH 5μm 150x4.6 mm (HPLC Technology Macclesfield, UK) with acetonytrile (HPLC grade) and formic acid (98-100 %), both of Merck Darmstadt. NMR spectra were obtained on VXR 300 NMR spectrometer (Varian, Palo Alto, CA, USA).

Procedure

Amlodipine benzensulphonate (0.5 g) and bis(2-ethylhexyl) phthalate (60 mg) were heated under reflux conditions in methanol (4 ml) for 24 hrs. Solvent was then removed under reduced pressure, and the dry residue was analyzed. Analyses were performed after 0 and 24 hrs.

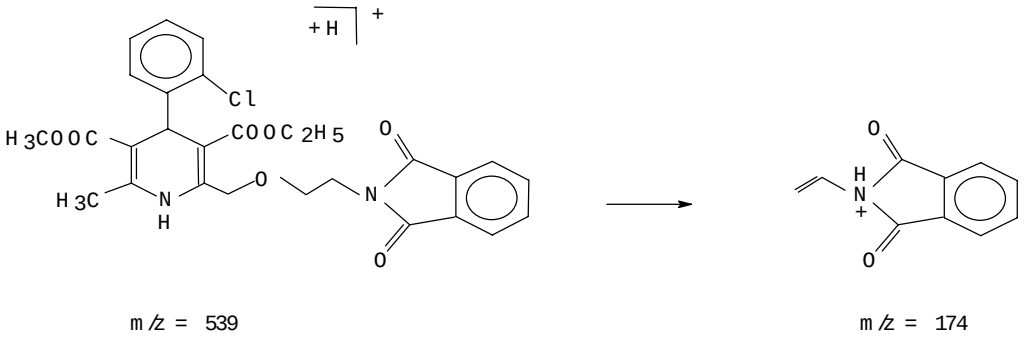
Analyses

Chromatography: Linear gradient (80% A to 30% A in 5.5 min; A - 70% acetonitrile and 0.1% formic acid, B - acetonitrile and 0.1% formic acid), flow 1.5 ml/min; samples (100 μL) were dissolved in mobile phase A, concentration 10 mg/ml; standard concentrations were 200, 2 and 0.7 ng/ml, respectively.

Mass spectrometry:

The HPLC was connected to a tandem mass spectrometer via an APCI interface. The vaporiser temperature was 500°C and the capillary was held at 200°C. The corona current was 5 μA. Parameters for CID were: collision gas argon at pressure of 2.0 mTorr (1mTorr=133.3 Pa), collision energy 25 eV. Selected reaction monitoring was carried out on $(M+H)^+$ ion at m/z 539, going to m/z 174 (Scheme II). Quantisation was performed by comparison of the fragmentogram at m/z 174 for standard of phthalimido derivative **3,** and samples. Limit of quantisation was 10 ppb.

Scheme 2. Fragmentation scheme of $(M+H)^+$ ion m/z 539 to daughter ion m/z 174.



RESULTS

Content of the impurity **3** in the initial reaction mixture was found to be below limit of quantisation (i.e. below 10 ppb), while after 24 hrs of heating under reflux condition it was found to be 309 ppb.

CONCLUSIONS

Results show that bis(2-ethylhexyl) phthalate (**2**) slowly reacts under reflux conditions with amlodipine besylate **1** to form traces of phthalimido derivative **3**, and thus explain the origin of the unexpected impurity in our synthetic product: the impurity **3** is

formed by reaction of bis(2-ethylhexyl) phtalate, released from plastic packaging and/or process equipment materials. It can be classified as a secondary impurity.

These findings are important for general understanding of possible origins of impurities in organic synthesis products since they clearly demonstrate that packaging and process equipment materials can be a source of impurities directly by elution, or indirectly by reactions of eluted components. In addition, it is evident that when considering impurities in a product, reactions at trace level should be taken into account, i.e. the chemistry of side reactions and reactions among trace components should be considered in contrast to the general synthetic approach primarily focused to reaction yields of desired products. This is of particular importance for secondary impurities elaboration.

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Povzetek

Obravnavani so potencialni viri nečitoč v produktih organske sinteze, pri čemer je uvedeno razlikovanje med primarnimi in sekundarnimi nečistočami. Z raziskavo izvora nečistoče **3** v amlodipin besilatu **1** smo pokazali, da lahko plastični materiali, uporabljani za embalažo ali procesno opremo, predstavljajo dodatni vir nečistoč. Raziskava je namreč pokazala, da nečistoča **3** nastane z reakcijo med amlodipin besilatom **1** in bis(2-etilheksil) ftalatom (**2**), ki se eluira iz plastične embalaže, v kateri so skladiščena topila za sintezo amlodipina. Za raziskavo je bila razvita selektivna in občutljiva HPLC-MS/MS metoda.