Identification and control of unspecified impurity in trimetazidine dihydrochloride tablet formulation

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Abstract: Trimetazidine dihydrochloride is an anti-ischemic metabolic agent which is used as drug for angina pectoris treatment. The drug substance monograph is available in European Pharmacopoeia and British Pharmacopoeia, while the drug product monograph is not available in any of the pharmacopoeias. During development of trimetazidine dihydrochloride tablet formulation, we found increase of an unspecified impurity during preliminary stability study. The unspecified impurity was identified by high performance liquid chromatography coupled with mass spectrometry (LC-MS) and the molecular weight obtained was matching with the molecular weight of N-formyl trimetazidine (m/z 295). Further experiments were performed to confirm the suspected result by injecting the impurity standard and spiking formic acid into the drug substance. The retention time of N-formyl trimetazidine was similar to the unspecified impurity in drug product. Even spiking of formic acid into drug substance showed that the suspected impurity increased with increasing concentration of formic acid. The proposed mechanism of impurity formation is via amidation of piperazine moiety of trimetazidine by formic acid which present as residual solvent in tablet binder used in the formulation. Subsequently, the impurity in our product was controlled by choosing the primary packaging which could minimize the formation of impurity.

1 Introduction

Trimetazidine (1-(2,3,4-trimethoxybenzyl)piperazine) dihydrochloride (Figure 1) is an anti-ischemic metabolic agent used for treatment of angina pectoris with no vasodilator properties[1]. The drug’s mechanism of action is through inhibition of long-chain 3-ketoacyl CoA thiolase activity, thus inhibiting the fatty acid oxidation and stimulating glucose utilization[2]. Clinical studies show that anti-ischemic effect of trimetazidine is not associated with myocardial oxygen consumption which affects the heart rate and systolic blood pressure[1].

Figure 1. Chemical structure of trimetazidine dihydrochloride
The monograph of trimetazidine dihydrochloride drug substance is available in European Pharmacopoeia (EP) and British Pharmacopoeia (BP). However, currently there are no monographs available for the drug tablet formulation; hence it is considered as non-compendial product. In the drug substance monographs, there are nine specified impurities (impurity A – I)[3,4]. None of the impurities specified in drug substance monograph were observed during drug product development as most of them were process-related impurities owing to the route of synthesis. Preliminary stability studies of the drug product were performed at 30 °C, 75% RH and 40 °C, 75% RH for one month and showed that one unspecified impurity at relative retention time (RRT) 1.2 with respect to trimetazidine was increasing with time. The impurity level increased up to 0.24% in 4 weeks under accelerated stability condition (40 °C, 75% RH). This value has exceeded the identification threshold as per International Conference on Harmonisation (ICH) guideline (0.2% for maximum daily dose of >10 mg – 2 g)[5]. Hence, the identification of this unspecified impurity using LC-MS was initiated and measures were taken to control it in our drug product.

2 Experimental

2.1 Chemicals, reagents and samples
Trimetazidine dihydrochloride drug substance was purchased from Bachem SA (Vionnaz, Switzerland) and the tablet formulation (35 mg/tablet) was developed in-house by Dexa Medica. Trifluoroacetic acid spectroscopy grade, methanol HPLC grade, ortho-phosphoric acid 85% AR grade and formic acid 98-100% AR grade were commercially obtained from Merck (Germany). Ion-pairing agent sodium 1-heptanesulfonate was procured from Tokyo Chemical Industry (TCI) Co. Ltd. Water used for chromatography was prepared using Milli-Q water purification system from Merck Millipore. N-formyl trimetazidine impurity standard was procured from Simson Pharma (Mumbai, India).

2.2 LC-MS conditions
Identification of the unspecified impurity was performed on Shimadzu LCMS-2010 series equipped with Photo Diode Array (PDA) detector. The method was based on the publication of Y Jiao and co-workers[6] with modifications in column length and flow rate. Zodiac-C_{18} column with dimensions of 250 mm x 4.6 mm; 5 µm particle size was utilized at 40 °C. The mobile phase consisted of water adjusted to pH 2 with trifluoroacetic acid : methanol = 6:4 (v/v). Flow rate was 0.8 mL/min in an isocratic mode and injection volume was 50 µL. The UV chromatogram was extracted at 240 nm. MS spectrum was scanned from m/z 100-450 using Atmospheric Pressure Chemical Ionization (APCI) probe in positive ion mode.

2.3 HPLC-UV conditions
Confirmation of identification result using HPLC-UV was performed on Waters alliance e2695 separation module equipped with Waters 2489 UV/Vis detector and Empower 2 data handling system. The method was directly adopted from monograph of trimetazidine dihydrochloride drug substance[3,4] utilizing Atlantis dC-18 column (150 mm x 4.6 mm; 5 µm particle size).

2.4 Sample preparation
For LC-MS analysis, one tablet of trimetazidine dihydrochloride was transferred into 50 mL volumetric flask (previously rinsed with methanol and dried), added 15 mL of methanol and sonicated for 15 minutes to disintegrate the tablet, diluted to volume with water and centrifuged at 6000 rpm for 15 minutes. Later, 7.5 mL of the supernatant was pipetted and transferred into 50 mL volumetric flask, diluted to volume with water and filtered through 0.2 µm PTFE membrane filter. In HPLC-UV analysis, 200 mg of drug substance was weighed and transferred into 50 mL volumetric flask, added 30 mL of water and sonicated for 5 minutes until dissolved. Formic acid solution was spiked into three sample solutions separately to obtain three concentration levels (50, 100, 150 ppm), then each solution was diluted to volume with water and mixed homogenously.
3 Results and discussion

3.1 Identification of unspecified impurity by LC-MS

The stability sample of trimetazidine dihydrochloride tablet was analyzed using LC-MS with conditions as previously mentioned to identify the unspecified impurity by its molecular weight. The target impurity eluted after the main peak with retention time (RT) at about 5.5 min. Figure 2 and 3 shows the typical chromatogram of sample and mass spectrum of unspecified impurity obtained in the analysis.

![Figure 2. Typical chromatogram of sample in LC-MS analysis](image1.png)

![Figure 3. Mass spectrum of unspecified impurity](image2.png)

The mass spectrum, recorded in positive ion mode, of the unspecified impurity exhibited a base peak at \(m/z\) about 295 (M+H) which suggested that the molecular weight of impurity was 294. Compared to trimetazidine (MW 266), the molecular weight of the impurity is 28 amu more than the main compound. This mass difference corresponds to carbonyl (C=O) functional group which can attach to the piperazine moiety of trimetazidine forming the \(N\)-formyl impurity whose structure is shown in Figure 4 below.

![Figure 4. Chemical structure of \(N\)-formyl trimetazidine](image3.png)

3.2 Confirmation of identification result by HPLC-UV

Based on its molecular weight, the unspecified impurity was suspected as \(N\)-formyl trimetazidine. To further confirm the identity of impurity, we purchased the impurity standard and compared the retention
time of impurity with the standard in HPLC. Moreover, three concentration levels (50, 100, 150 ppm) of formic acid was spiked into the sample to observe the correlation between impurity formation and formic acid concentration as the acid could react with trimetazidine to form N-formyl trimetazidine\cite{7}. The chromatogram in Figure 5a showed that the retention time of the unspecified impurity was matching with N-formyl trimetazidine standard at around 10 min. The other experiment (Figure 5b) demonstrated that the impurity increased along with increasing concentration of formic acid spiked into the drug substance.

![Figure 5](image)

**Figure 5.** Typical chromatograms of (a) sample and N-formyl trimetazidine standard, (b) spiking of formic acid into trimetazidine dihydrochloride drug substance in HPLC-UV analysis

### 3.3 Proposed mechanism of impurity formation

Increase of impurity level by spiking of formic acid suggested that the formation of N-formyl trimetazidine involved reaction between trimetazidine in the drug product with formic acid\cite{7}. After reviewing the list of excipients used in the formula, the tablet binder contains formic acid as residual solvent with limit of 0.5% (reported value 0.2%). The presence of formic acid in the tablet binder could react with the drug substance via amidation of piperazine moiety of trimetazidine forming the N-formyl impurity as shown in Figure 6.

![Figure 6](image)

**Figure 6.** Proposed mechanism of N-formyl trimetazidine formation
3.4 Control of impurity in finish product

Although the root cause of the impurity formation had been identified, the use of the tablet binder which contained formic acid was inevitable due to its critical function in the tablet formulation. The other solution was to control the impurity by minimizing its formation. In one of the optimization trials, we observed that primary packaging material selection might have impact on the impurity formation. Based on the data presented in Table 1, we concluded that polycellonium strip was the best primary packaging for the drug product.

Table 1. Impurity result of trimetazidine dihydrochloride tablet in three different packaging materials after stored in climatic chamber at 60 °C, 75% RH for one week

| Impurities       | PVC blister | PVDC blister | Polycellonium strip |
|------------------|-------------|--------------|---------------------|
| Impurity A       | ND          | ND           | ND                  |
| Impurity B       | ND          | ND           | ND                  |
| Impurity C       | ND          | ND           | ND                  |
| Impurity D       | ND          | ND           | ND                  |
| Impurity E       | ND          | ND           | ND                  |
| Impurity F       | ND          | ND           | ND                  |
| Impurity G       | ND          | ND           | ND                  |
| Impurity H       | ND          | ND           | ND                  |
| Impurity I       | ND          | ND           | ND                  |
| N-formyl trimetazidine | 0.28%   | 0.26%  | 0.22% |
| Total impurities | 0.28%       | 0.26%  | 0.22% |

*ND = not detected

4 Conclusion

The unspecified impurity in trimetazidine dihydrochloride tablet formulation was identified as N-formyl trimetazidine by means of LC-MS. Further experiments confirmed the identity of the impurity. The possible mechanism of the impurity formation was also proposed which involved reaction of the drug with residual formic acid present in tablet binder used in the formula. Finally, the impurity formation was controlled by choosing the best primary packaging material used for the drug product.

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