# Identification and structural elucidation of process impurities generated in the end-game synthesis of taranabant (MK-0364) via cyanuric chloride

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## ARTICLE INFO

## ABSTRACT

Taranabant (MK-0364) is a highly potent and selective cannabinoid-1 receptor (CB-1R) inverse agonist. It is being developed at Merck & Company to treat obesity. The chemical synthesis of MK-0364 drug substance involved the direct coupling of chiral amine and pyridine acid side chains mediated by cyanuric chloride. Four major process impurities were observed and characterized using high performance liquid chromatography (HPLC) coupled with ultraviolet (UV) and electrospray ionization (ESI) mass spectrometry (MS) detectors. The exact mass data was used for structural elucidation which suggests that the impurities are derivatives of cyanuric chloride formed in the coupling step. Owing to the reactive nature of these impurities, an interesting degradation phenomenon was observed during stability testing of MK-0364 drug substance when stored at 40 °C/75% RH and 25 °C/60% RH conditions. Degradation pathways were proposed to explain the changes observed in the HPLC impurity profile. Forced degradation experiments were also conducted to confirm the degradation pathways and assess the stability of the impurities. Finally, the complete stability data of the bulk drug are reported to support the hypothesis.

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## 1. Introduction

Taranabant (MK-0364, N-[(1S,2S)-3-(4-chlorophenyl)-2-(3-cyanophenyl)-1-methylpropyl]-2-methyl-2-{[5-(trifluoromethyl)pyridin-2-yl]oxy}propanamide), is a highly potent and selective cannabinoid-1 (CB-1) inverse agonist, which is currently under investigation at Merck Research Laboratories for the treatment of obesity [1]. The rationale supporting the drug discovery was that agonists at the G-protein coupled CB-1R, such as tetrahydrocannobinol (THC), are known to stimulate appetite in humans [2]. THC has been approved in the United States for the treatment of AIDS- or cancer-associated anorexia [3]. Thus, it is anticipated that a CB-1R antagonist or inverse agonist will be effective in lowering food intake and thus in the treatment of obesity. Preclinical studies have shown that its efficacy is correlated with appropriate CB-1R occupancy in the brain in certain rodent species [4].

An elegant, practical, and economically viable chemical synthesis route was developed [5] to produce large quantity of MK-0364

drug substance in order to support the development of the program. In the final step of the chemical process, illustrated in Scheme 1, the coupling reaction between the chiral amine **1** HCl salt and the pyridinyl acid **2** was carried out in a mixture of acetonitrile and *N*-methylmorpholine (NMM) to afford crude MK-0364. The major improvement for the coupling reaction was the substitution of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) with cyanuric chloride which is of low cost and easy availability as the coupling reagent [6]. The product, crude API, was subsequently crystallized in a mixture of n-heptane and isopropyl acetate (IPAc) to upgrade chiral purity from 94 to >98 ee% and then recrystallized from IPAc and n-heptane to produce anhydrous MK-0364 drug substance. Among many problems encountered during the process development, the most challenging one was that multiple impurities arisen from the coupling reaction owing to the high reactivity of cyanuric chloride toward other reagents. These impurities not only have a significant impact on the final purity and stability of the drug substance, but also complicate the subsequent enantiomeric excess (ee) upgrade process and the final crystallization. Without reducing the impurities below a certain threshold, the ee upgrade can be difficult to achieve and the crystal growth is hindered. Therefore, it is important to have an analytical method capable of accurate identification and quantification of the impurities.

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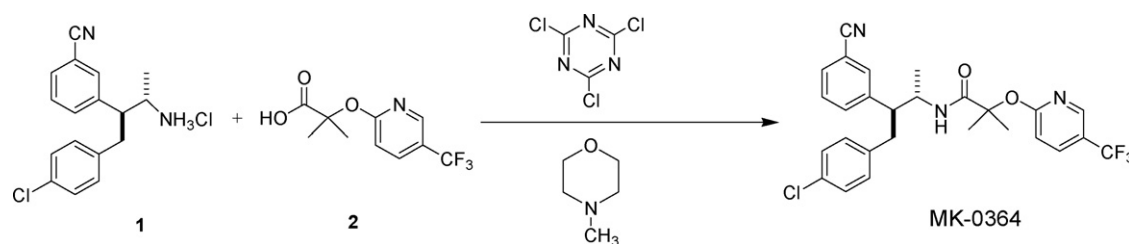
**Scheme 1.** End-game synthesis of MK-0364 drug substance—coupling reaction via cyanuric chloride.

The HPLC analysis showed that five impurities were present in the first pilot plant batch of MK-0364 drug substance. Four of them, referred as Compounds **A**, **B**, **C**, and **D** in this paper, were new impurities generated in the end-game. The last impurity, the des-cyano analog of MK-0364, was a known impurity which originated from the previous step. Since the level of new impurities was approaching or exceeding the 0.10% identification threshold according to the International Conference on Harmonization (ICH) guidelines [7], LC/MS analysis was performed for structure identification. Preliminary results suggested that Compounds **A**, **B**, **C**, **D** were various cyanuric chloride adducts of the chiral amine sidechain.

Following the release of the batch, stability studies were initiated under accelerated (40 °C/75% RH) and long term (25 °C/60% RH) conditions. At the 1 month time point under the accelerated conditions, we observed that the HPLC impurity profile had changed significantly, with Compound **B** nearly doubling, and a new impurity (Compound **E**) observed at 0.10%. Since the chemical stability can significantly impact the quality and safety of drug substance, this result prompted an immediate investigation.

We report herein our efforts in determining the identity and quantity of the process impurities generated in the coupling reaction. In addition, this paper describes in detail the use of results from LC/MS/UV analysis, forced degradation studies, process information, and the eventual long-term stability data to elucidate the mechanistic pathways and determine the stability for the process impurities.

## 2. Experimental

### 2.1. Chemicals

The common chemicals and reagents used in the studies were purchased from Sigma–Aldrich Chemical Company (Milwaukee, WI, USA) and used as received. Cyanuric chloride was purchased

from Degussa Corporation. The pyridine acid **2** (Scheme 1) was manufactured by DSM Chemicals (Netherlands) and the chiral amine **1** side chain was prepared according to the procedure described recently [5].

### 2.2. HPLC conditions for impurity profile analysis

The HPLC system consisted of an Agilent model 1100 liquid chromatograph that included an auto-injector, a quaternary pump, and a photo-diode-array (PDA) detector. Sample solutions were automatically injected onto a Zorbax Eclipse XDB-C18 column (Waters) with 25 cm × 4.6 mm i.d. dimension and 5 μm particle size, maintained at 25 °C. The mobile phase consisted of 0.1% $H_3PO_4$ in water (Solvent-A) and HPLC grade acetonitrile (Solvent-B) at a flow rate of 1.5 ml/min. The separation was achieved employing a linear gradient from 35% (v/v) Solvent-B to 80% (v/v) Solvent-B in 20 min, then holding at 80% (v/v) Solvent-B over 10 min. Detection by UV was set at 220 nm and PDA spectra were collected when possible. Samples were prepared at approximately 0.2 mg/ml in water/acetonitrile 50/50 (v/v) mixture, unless otherwise stated, and the injection volume was 10 μl.

### 2.3. Validation of the HPLC method

The HPLC method described above was validated with respect to linearity, precision/repeatability, limit of detection (LOD), and limit of quantitation (LOQ). The linearity of the method was demonstrated over the concentration range from 0.25 mg/ml (125% of the parent level) to 0.0001 mg/ml (0.05% of the parent level) of MK-0364. The correlation coefficient ($R^2$) was >0.9999. The method precision/repeatability was assessed based on injection precision. The %RSD of an impurity at 0.12% over 2 sample preparations and 6 injections was <5.0%. LOD of the method was 0.02% based on a minimum signal to noise ratio (S/N) of 3 for the MK-0364 main peak.
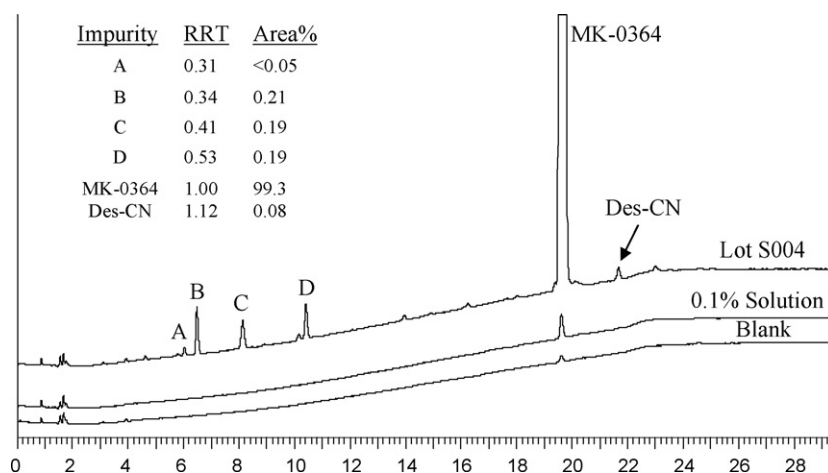


| Impurity | RRT | Area% |
|----------|------|-------|
| A | 0.31 | <0.05 |
| B | 0.34 | 0.21 |
| C | 0.41 | 0.19 |
| D | 0.53 | 0.19 |
| MK-0364 | 1.00 | 99.3 |
| Des-CN | 1.12 | 0.08 |

**Fig. 1.** HPLC impurity profile of MK-0364 drug substance (Lot S004) at delivery.
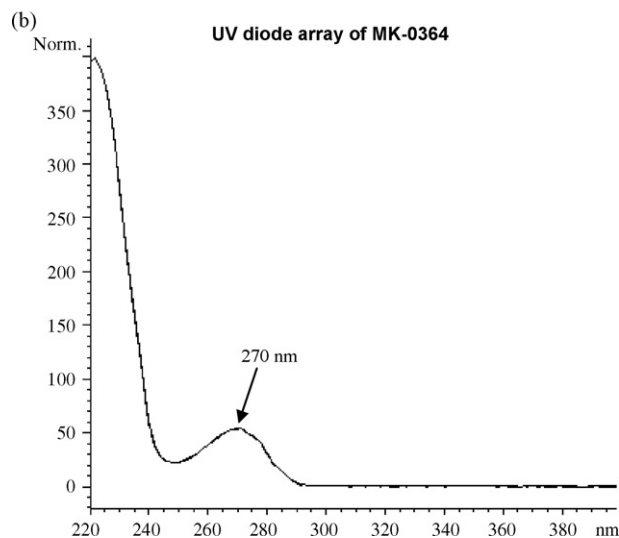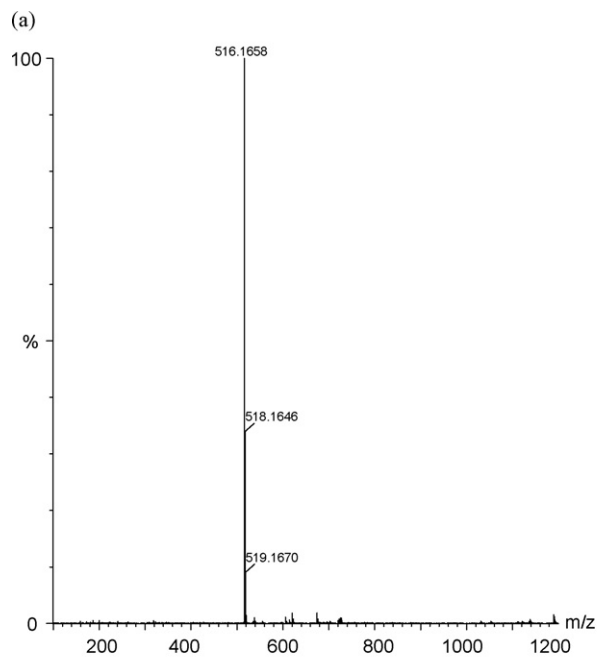
(a)



(b)

**UV diode array of MK-0364**



**Fig. 2.** Mass and UV spectra of Taranabant (MK-0364): (a) mass spectrum and (b) UV diode array spectrum.

LOQ was 0.05% based on a minimum S/N of 10 for the MK-0364 main peak.

### 2.4. HPLC mass spectrometry conditions for impurity identification

The HPLC conditions for LC/MS analysis matched those described above except the mobile phase A was 0.1% formic acid. The mass spectrometer was a Finnigan TSQ 7000 equipped with an electrospray interface connected in series after the photo-diode-array detector. The electrospray voltage was 4.5 kV. The sheath and auxiliary gases were nitrogen at 60 and 30 units, respectively. The interface capillary was operated at 250 °C and the manifold temperature was 70 °C. In full scan mode, the mass ranges were from 0.5 to 2.5 times the mass of the compound of interest at an acquisition time of 2 s per spectrum. The resolution in the first quadrupole, Q1, was decreased resulting in a parent ion transmission of 4.0 amu full width at half height (FWHH). The product ion spectrum was also acquired at the rate of 2 s per spectrum. The interface cap-

illary and tube lens voltage were optimized by injection of the compound of interest into the protic mobile phase carrier. All MS measurements were made in the positive ion mode in the range of 100–1000 amu.

### 2.5. Stability conditions

Samples were packaged in double poly bags in a fiber drum and stored in the 40 °C/75% RH (accelerated) and 25 °C/60% RH (long term) chambers, respectively, mimicking manufacture packaging and storage conditions. The testing frequency was set as the initial, 1-, 2-, 3-, and 6-month for the accelerated study and the initial, 3-, 6-, 9-, 12-, 18-, 24-, and 36-month for the long-term study.

### 2.6. Forced degradation procedures

A freezer control sample of MK-0364 was dissolved in acetonitrile/water/$H_3PO_4$ 50/50/0.1% (v/v) and the sample solution was subject to thermal stress at 40 °C for 36 h followed by HPLC analysis. Another aliquot of the solid sample was heated in a 100 °C oven for 12 h, then dissolved in acetonitrile/water 50/50 (v/v) and injected onto HPLC for impurity profile analysis.
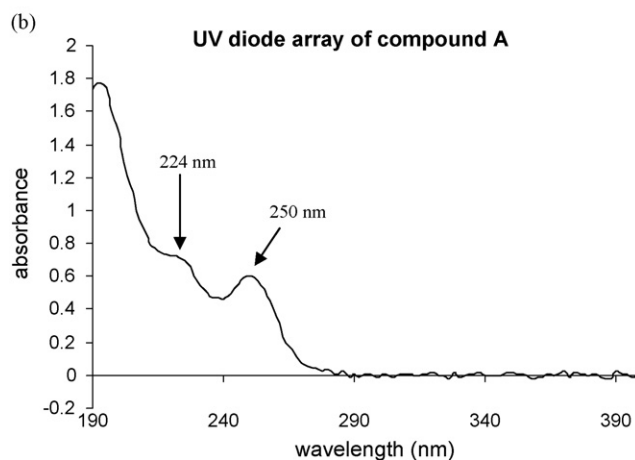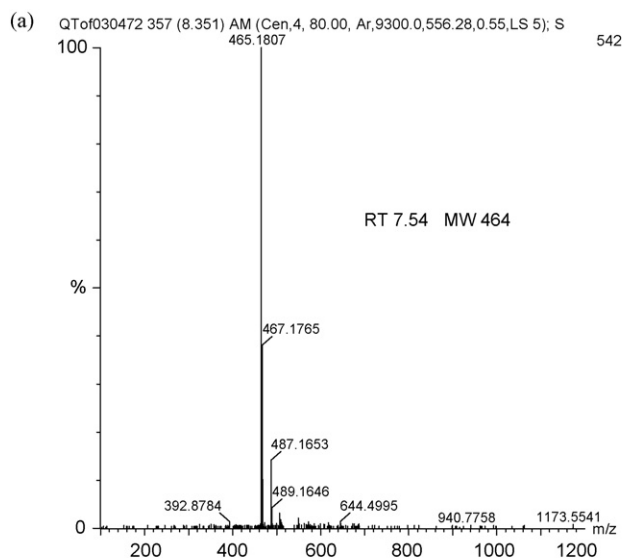
(a)



(b)

**UV diode array of compound A**



**Fig. 3.** Mass and UV spectra of compound **A**: (a) mass spectrum and (b) UV diode array spectrum.

**Fig. 4.** Mass and UV spectra of compound **B**: (a) mass spectrum and (b) UV diode array spectrum.
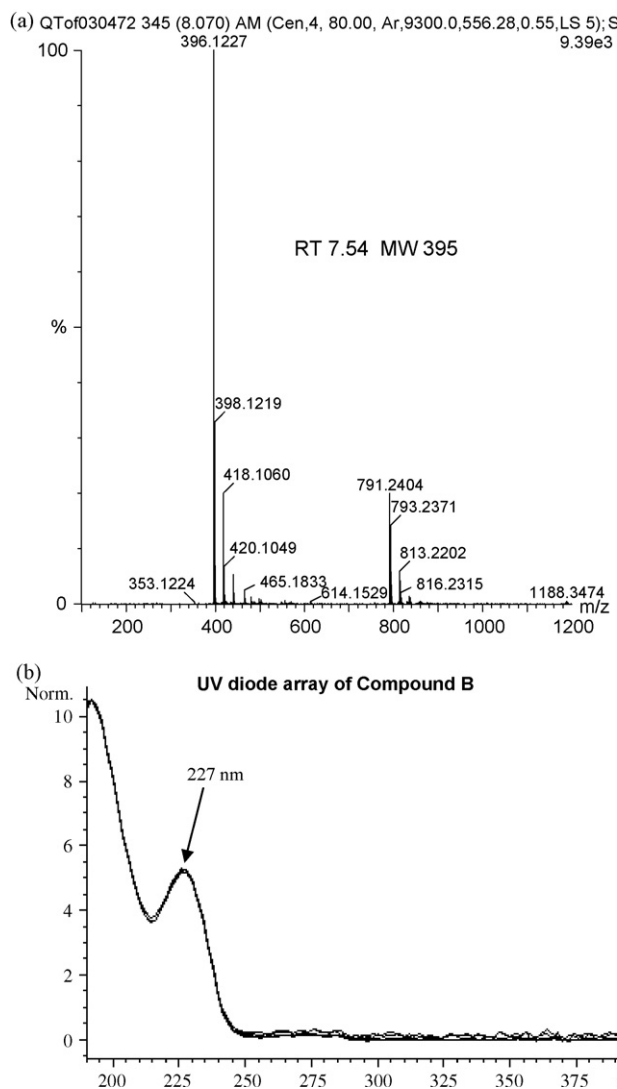
**Fig. 5.** Mass and UV spectra of compound **C**: (a) mass spectrum and (b) UV diode array spectrum.

## 3. Results and discussion

### 3.1. HPLC analysis of drug substance at delivery

Fig. 1 shows the HPLC chromatogram of the MK-0364 drug substance (Lot S004) at the release testing. In addition to the main peak for MK-0364, five small impurity peaks were observed. The level of these impurities by area% and their relative retention time (RRT) to the MK-0364 main peak are shown in Fig. 1. Four of them, referred to as Compounds **A**, **B**, **C**, and **D** in this paper, were new impurities arising from the coupling step. The last eluted impurity, the des-cyano analog of MK-0364, is a known impurity originating from the upstream synthesis and is not the subject of discussion in this paper. Since the level of the new impurities (except for Compound **A**) exceeded the 0.10 area%, according to the ICH guidelines, structural identification was required for the unknown impurities prior to qualifying the material for clinical use.

### 3.2. Impurity identification and structure elucidation

The mass and UV spectra of MK-0364 drug substance as well as Compounds **A**, **B**, **C**, and **D** are shown in Figs. 2–6. The HPLC RRT, UV
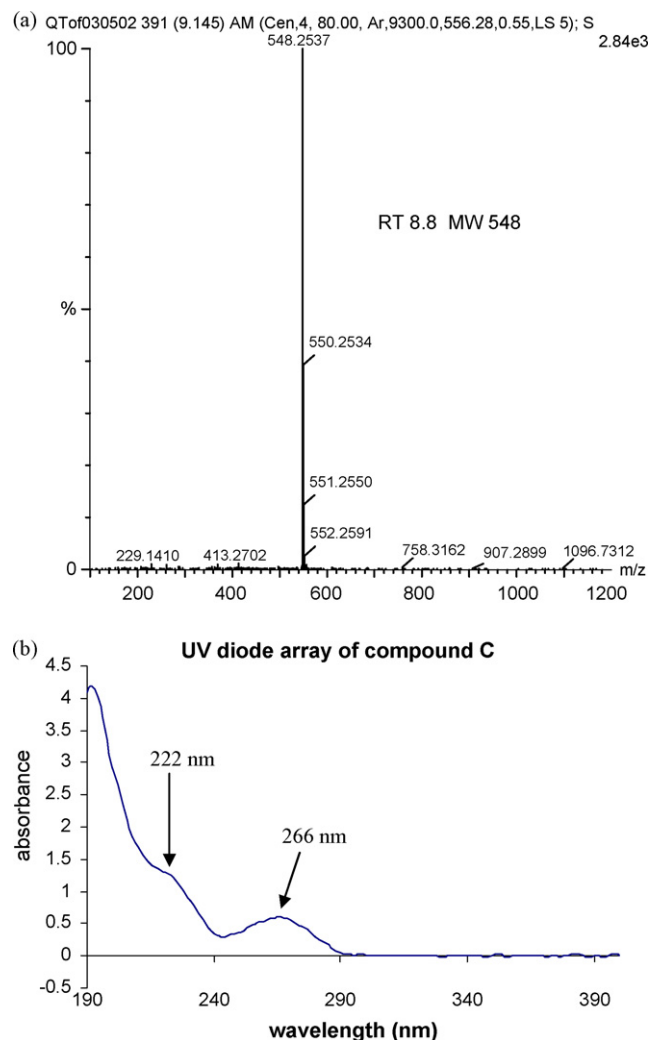
$\lambda_{max}$ values, calculated and measured exact masses, and proposed structures are summarized in Table 1.

Characterization of the impurities was carried out employing LC/UV, LC/MS and MS/MS analysis and structural elucidation primarily based on the exact mass by MS which was consistent with complementary analytical results and process information. For example, RRT value is indicative of molecular structure and properties such as hydrophobicity and size of impurity relative to the main component. In reversed phase chromatographic separation, lower RRTs correspond to less hydrophobic and/or smaller molecular weight compounds, often leaving clues about the presence of a protonatable functional group (e.g. amine) in the structure. Comparison of the UV spectra also provides semi-qualitative evidence as to whether the impurity is an analog of the main component or an entirely different compound. Similarity in the UV spectral pattern indicates similarity in the structure and functional groups attached. A shift in $\lambda_{max}$ to higher wavelength indicates an extension of conjugation. Ultimately, LC/MS and MS/MS analysis yields definitive information on molecular weight, isotopic, and fragmentation patterns. Combining the cumulative analytical results with the process information allowed us to elucidate structures which were in agreement with the rest of the findings.

Compound **B**, due to its relatively high level, was the first target for structure determination. The ESI mass spectrum of Compound **B** (Fig. 4a) displayed a molecular ion peak [M+H]$^+$ at 396 amu,

**Table 1**
Summary of the impurities generated in the coupling reaction.

| Compound name, HPLC relative retention time (RRT) and UV $\lambda_{max}$ | Calculated and measured masses for [M+H] ions by ESI mass spectrometry | Proposed structure |
|---|---|---|
| Compound **A**, RRT 0.31, $\lambda_{max}$: 224, 250 nm | Calculated: 465.1806; measured: 465.1807 |  |
| Compound **B**, RRT 0.34, $\lambda_{max}$: 227 nm | Calculated: 396.1227; measured: 396.1227 |  |
| Compound **C** (positively charged), RRT 0.41, $\lambda_{max}$: 222, 266 nm | Calculated: 548.2541; measured: 548.2537 |  |
| Compound **D**, RRT 0.53, $\lambda_{max}$: 227 nm | Calculated: 414.0888; measured: 414.0887 |  |
| Compound **E**, RRT 0.49, $\lambda_{max}$: 224, 250 nm | Calculated: 534.2378; measured: 534.2379 |  |
| Taranabant (MK-0364), RRT 1.00, $\lambda_{max}$: 270 nm | Calculated: 516.1666; measured: 516.1658 |  |

**Table 2**
HPLC impurity profile of MK-0364 (Lot S004) stability samples at 1-month.

| RRT | Component | Area% at $t = 0$ | Area% 1-mo 25/60 | Area% 1-mo 40/75 | Changes |
|---|---|---|---|---|---|
| 0.31 | Compound **A** | <0.05% | <0.05% | 0.08% | Increased |
| 0.34 | Compound **B** | 0.21% | 0.39% | 0.48% | Increased |
| 0.41 | Compound **C** | 0.19% | 0.16% | 0.09% | Decreased |
| 0.49 | Compound **E** | ND < 0.02% | 0.06% | 0.10% | New Imp |
| 0.53 | Compound **D** | 0.19% | 0.06% | ND < 0.02% | Disappeared |
| 1.00 | MK-0364 | 99.3% | 99.2% | 99.1% | Unchanged |
| 1.10 | Des-cyano Imp | 0.08% | 0.08% | 0.08% | Unchanged |
| Total impurities | | 0.66% | 0.75% | 0.82% | Increased |

**Fig. 6.** Mass and UV spectra of compound **D**: (a) mass spectrum and (b) UV diode array spectrum.

**Table 3**
Thermal stress of MK-0364 in solution (0.1% $H_3PO_4$/acetonitrile 50/50, v/v).

| RRT | Component | Area% before heating | Area% 40 °C for 36 h | Changes |
|-----|-----------|---------------------|---------------------|---------|
| 0.31 | Compound **A** | <0.05% | 0.05 | Slightly increased |
| 0.34 | Compound **B** | 0.26 | 0.54 | Doubled |
| 0.41 | Compound **C** | 0.17 | 0.20 | Slightly increased |
| 0.49 | Compound **E** | 0.06 | 0.06 | Unchanged |
| 0.53 | Compound **D** | 0.15 | ND < 0.02 | Disappeared |
| 1.00 | MK-0364 | 99.2% | 99.0% | Unchanged |
| 1.10 | Des-CN | 0.08 | 0.09 | Unchanged |
| Total impurities | | 0.72 | 0.94 | Increased |

by HPLC. A significant change in the impurity profile was observed. First, Compound **A** increased from the previous non-quantifiable level (<0.05%) to 0.08%. The level of Compound **B** was more than doubled from 0.21% to 0.48%. Compound **C** dropped from 0.19% to 0.08%, and Compound **D** completely disappeared from 0.19% to non-detectable level (ND < 0.02%). Surprisingly, a new impurity, Compound **E** (RRT 0.49), was detected at 0.10%. The total impurities increased by 0.1% while the MK-0364 area% remained unchanged. The problem was serious enough to call for an immediate investigation.

A stability sample under the long-term condition (25 °C/60% RH) was also withdrawn for HPLC analysis. When the two sets of data were compared (Table 2), it was clear that the changes in the impurity profile occurred under both storage conditions, but more rapid under 40 °C/75% RH. The stability sample was further analyzed using LC/MS to determine the structure of the new impurity, Compound **E** (Table 1). Based on the UV (Fig. 7a) and mass (Fig. 7b) spectral information, Compound **E** is believed to be the degradation product of Compound **C** after losing the methyl group. This is consistent with the HPLC elution order of the two impurities. Compound **C** (RRT 0.41), although with an extra methyl group, elutes earlier than Compound **E** (RRT 0.49) due to the quaternary ammonium (positively charged) on the triazine moiety. Identification of Compound **E** provided crucial clues that helped determine the cause for other impurity changes as the degradation pathways and the rate of the degradation were further examined.

### 3.4. Forced degradation study

Forced degradation (stress) studies are carried out under even harsher conditions than those used for accelerated stability testing. Generally performed early in the drug development process, the natural degradation rate of drug substance is increased by the application of additional stress such as acid and base hydrolysis, peroxide oxidation, thermal stress and photostability. The results can be used to understand the byproducts and pathways that are necessary to develop stability indicating methods [8]. No significant degradation was observed in a previous MK-0364 sample (99.9% purity by HPLC) when subjected to the stress condition indicating that pure MK-0364 is chemically stable. Low level of degradation products were baseline resolved from the main component confirming the stability indicating power of the method. LC/MS results (not reported here) suggested that the byproducts were not related to cyanuric chloride.

For the purpose of this investigation, since the compound was low in moisture content (water <0.1%), the focus was on measuring the effect of thermal stress on the impurity profile. A sample of MK-0364 (Lot S004) was subjected to thermal stress in both solution (acetonitrile/water/$H_3PO_4$ 50/50/0.1%, v/v) and solid state, followed by HPLC analysis.

Table 3 shows the impurity profile data of thermally stressed solution after 36 h at 40 °C. Most noticeably, Compound **B** was more than doubled from 0.26% to 0.54%; Compound **A** increased

which is less than that of MK-0364 (516 amu). The isotopic pattern [M+2] clearly indicated the presence of one chloride in the structure, raising the possibility that its origin is the chiral amine **1** side chain. The exact mass at 395 amu, an odd number, indicated an odd number of nitrogen in the structure. Using high resolution mass data (Table 1), an accurate molecular formula was calculated as $C_{20}H_{18}ClN_5O_2$, which led to the structural determination for Compound **B**. Compound **B** was formed by hydrolysis of the intermediate compound generated by the reaction of the chiral amine **1** with cyanuric chloride. The proposed structure was consistent with its low HPLC retention (RRT at 0.34) as it is smaller in size and more polar than MK-0364 due to the triazine moiety and hydroxyl functional groups in the structure.

Employing similar logic and rationale, structures for Compounds **A**, **C**, and **D** were elucidated. Like Compound **B**, they are various activation products of chiral amine **1** by cyanuric chloride that are either partially or completely hydrolyzed by water or displaced by NMM.

### 3.3. Stability study of the drug substance

Immediately after the release testing, stability studies of MK-0364 drug substance were initiated. At 1-month time point, a sample was pulled from the accelerated condition and analyzed
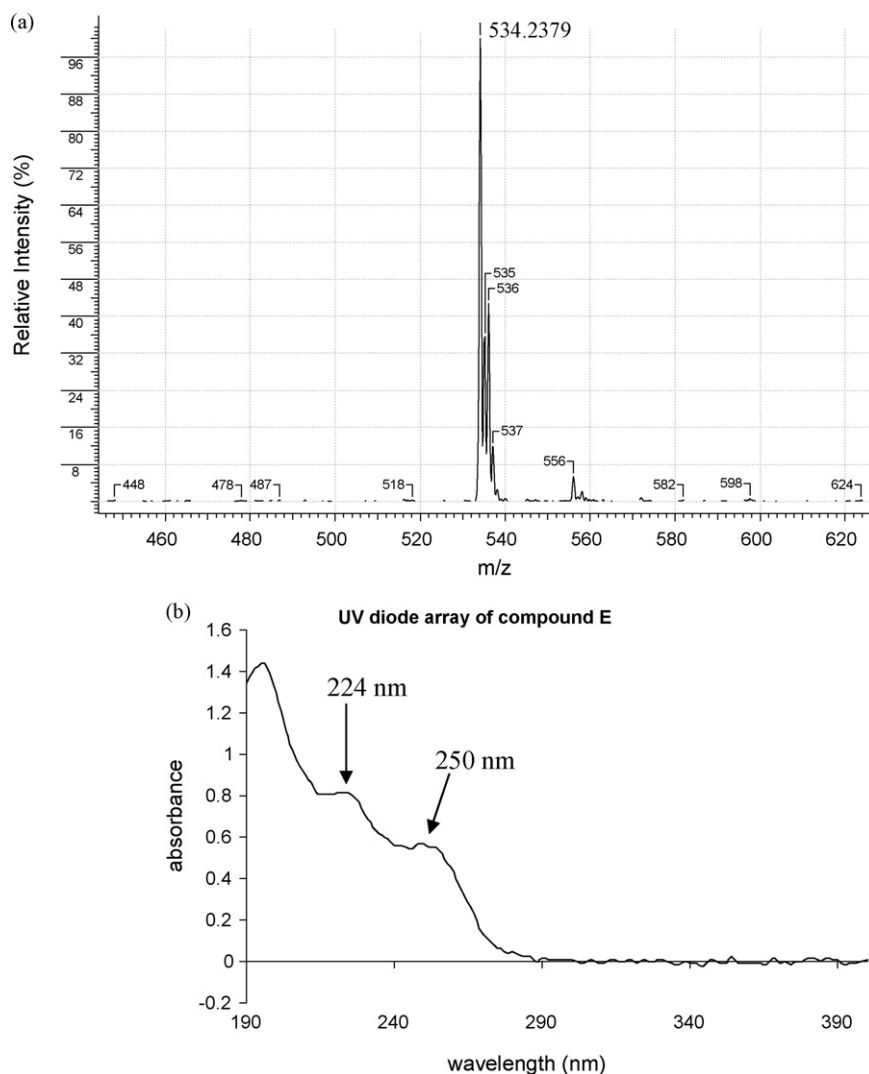
**Fig. 7.** Mass and UV spectra of compound **E**: (a) mass spectrum and (b) UV diode array spectrum.

very slightly; Compound **C** decreased rapidly from 0.19% to 0.09%; Compound **D** completely disappeared from 0.15% to ND; and Compound **E** remained unchanged at 0.06%. The area% of the MK-0364 main component only dropped slightly which was thought to be attributed to the differences in the UV response factors between the disappearing impurities and those formed. Results indicated that more Compound **B** was formed as the result of hydrolysis of Compound **D** as shown in Scheme 2a. Compound **D** contains a chloro-triazine moiety which is susceptible to acid-catalyzed hydrolysis [9]. Another possible degradation route is proposed in Scheme 2b for Compound **C** to form Compound **A** as reported by Kunishima in Ref. [10] for the decomposition of methylmorpholin-

ium salt. A good mass balance was achieved, namely the sum of the area% lost (0.29%) from Compounds **C** and **D** was in close agreement with the sum of the area% gained (0.31%) from Compounds **A** and **B**, supporting postulated pathways. Based on the results of stress studies, we predicted that once the source impurities are completely exhausted, the level of Compound **B**, the single largest impurity and degradation product, would plateau around 0.5%. Compound **E**, a neutral compound, was relatively stable in acidic condition and did not show further decomposition.

The thermally stressed solid sample followed similar degradation patterns as in the solution stress, but the rate of the reaction was different under the conditions investigated. Table 4 shows that after 12 h stress at 100 °C, Compound **B** was present at 0.34%, only an increase of 0.11% from the unstressed sample; Compound **E**, absent at $t = 0$, increased to 0.22%; Compound **A** increased very slightly to 0.05%; Compound **C** completely disappeared from 0.17% to ND; Compound **D** decreased from 0.17% to 0.05%. Results suggest that in the solid state where only a trace amount of moisture is available, Compound **D** still can be converted to Compound **B** due to the ease of hydrolysis of the chloro-triazine moiety. However, the main degradation was the thermolysis of Compound **C** (a quaternary amine cation) which is thermodynamically unstable due to steric hindrance of the methyl group, to Compound **E**, a neutral and thus more stable species (as shown in Scheme 2c). Once again, a close mass balance was obtained between the sum of the area% lost

**Table 4**
Thermal stress of MK-0364 in solid state.

| RRT | Component | Area% before heating | Area% 100 °C for 12 h | Changes |
|---|---|---|---|---|
| 0.31 | Compound **A** | <0.05% | 0.05% | Slightly Increased |
| 0.34 | Compound **B** | 0.23% | 0.34% | Increased |
| 0.41 | Compound **C** | 0.17% | ND < 0.02% | Disappeared |
| 0.49 | Compound **E** | ND < 0.02% | 0.22% | New Impurity |
| 0.53 | Compound **D** | 0.17% | 0.05% | Decreased rapidly |
| 1.00 | MK-0364 | 99.3% | 99.2% | Unchanged |
| 1.10 | Des-cyano Imp | 0.08% | 0.08% | Unchanged |
| | Total impurities | 0.70% | 0.75% | Increased |
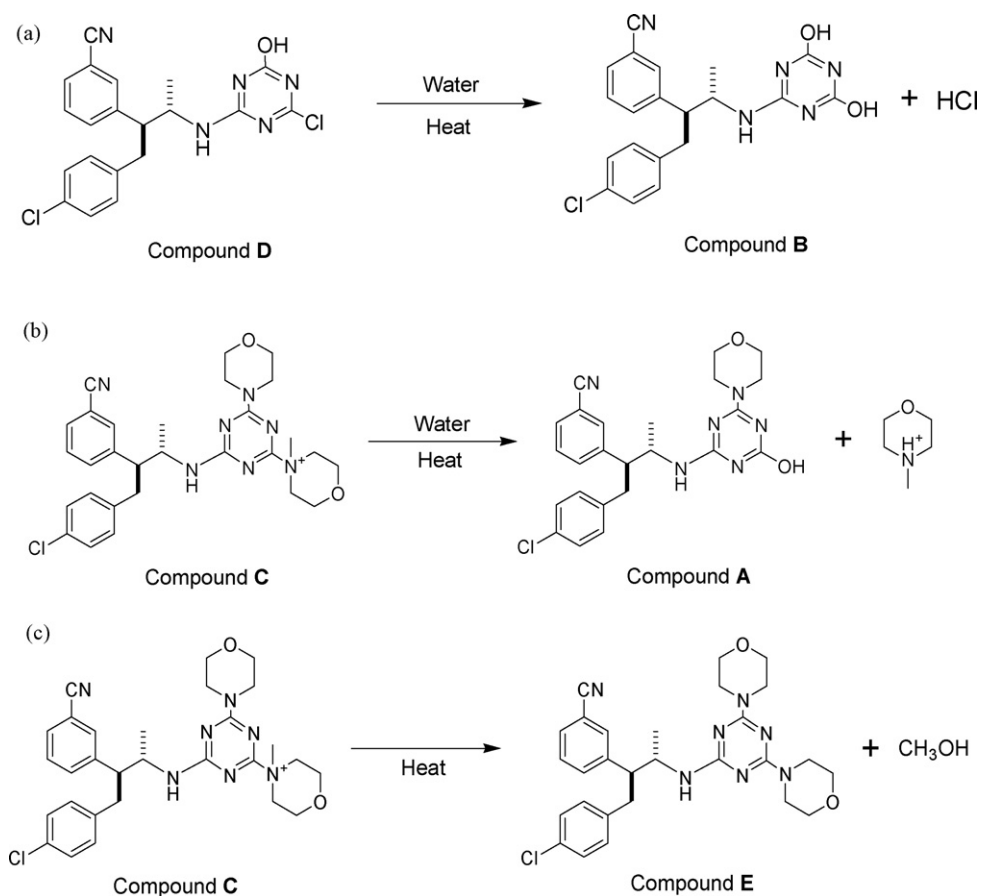
**Scheme 2.** Proposed impurity degradation pathways: (a) degradation of Compound **D** to Compound **B**; (b) degradation of Compound **C** to Compound **A**; (c) degradation of Compound **C** to Compound **E**.

**Table 5**
Accelerated stability data for MK-0364 (Lot S004).

| Stability environment: accelerated (40 °C/75% relative humidity) Container: doubled Nexus LDPE bags placed inside a fiberboard container | | | | | |
| --- | --- | --- | --- | --- | --- |
| Time points | Initial | 1 month | 2 month | 3 month | 6 month (end of study) |
| Impurity profile (area%) | | | | | |
| Total impurities | 0.66% | 0.82% | 0.81% | 0.76% | 0.80% |
| Compound **A** | NQ | 0.08 | 0.08 | 0.08 | 0.09 |
| Compound **B** | 0.21 | 0.48 | 0.47 | 0.47 | 0.49 |
| Compound **C** | 0.19 | 0.08 | 0.07 | 0.05 | NQ |
| Compound **E** | ND < 0.02 | 0.10 | 0.11 | 0.13 | 0.15 |
| Compound **D** | 0.19 | ND < 0.02 | ND < 0.02 | ND < 0.02 | ND < 0.02 |
| Des-CN product | 0.08 | 0.08 | 0.08 | 0.09 | 0.08 |

NQ = not quantifiable $0.02\% \leq NQ < 0.05\%$; ND = not detectable.

**Table 6**
Long-term stability data for MK-0364 (Lot S004).

| Stability environment: long-term (25 °C/60% relative humidity) Container: doubled Nexus LDPE bags placed inside a fiberboard container | | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Time points | Initial | 3 month | 6 month | 9 month | 12 month | 18 month | 24 month | 36 month (end of study) |
| Impurity profile (area%) | | | | | | | |
| Total impurities | 0.66% | 0.72% | 0.80% | 0.82% | 0.82% | 0.80% | 0.80% | 0.79% |
| Compound **A** | NQ | 0.05 | 0.06 | 0.06 | 0.06 | 0.07 | 0.06 | 0.06 |
| Compound **B** | 0.21 | 0.42 | 0.45 | 0.46 | 0.47 | 0.45 | 0.46 | 0.46 |
| Compound **C** | 0.19 | 0.13 | 0.12 | 0.11 | 0.11 | 0.09 | 0.08 | 0.07 |
| Compound **E** | ND < 0.02 | 0.08 | 0.09 | 0.10 | 0.11 | 0.12 | 0.12 | 0.12 |
| Compound **D** | 0.19 | NQ | NQ | NQ | NQ | ND < 0.02 | ND < 0.02 | ND < 0.02 |
| Des-CN product | 0.08 | 0.09 | 0.08 | 0.09 | 0.08 | 0.08 | 0.08 | 0.08 |

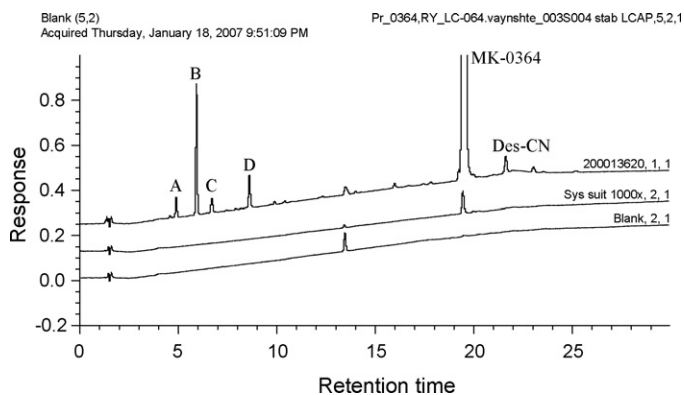NQ = not quantifiable $0.02\% \leq NQ < 0.05\%$; ND = not detectable.

**Fig. 8.** HPLC impurity profile of MK-0364 stability (25 °C/60% RH) at 36 months.

(0.29%) and the area% gained (0.33%) supporting our theory. Results of the solid state thermal stress studies allowed us to postulate that the decomposition is more rapid in the beginning, but would level off as the concentration of the source impurities decreases. Thus, it would not be necessary to change the storage condition from room temperature to refrigeration for the bulk MK-0364 drug substance if the degradation already started.

### 3.5. Complete stability data

We continued to closely monitor the degradation pattern in the stability samples after the initial investigation. At the end of the stability program, a complete stability data set for the accelerated and long-term conditions was obtained and reported in Tables 5 and 6. The HPLC impurity profile of the stability sample under 25 °C/60% RH at 36-month time point is shown in Fig. 8. We concluded that the degradation is merely a phenomenon of less stable impurities (Compounds **C** and **D**) converting to more stable ones (Compound **A**, **B** and **E**) through hydrolysis or thermolysis reactions in solid state. Degradation is slightly more rapid under accelerated condition as hydrolysis is generally facilitated by heat and humidity. Given the controlled levels of Compounds **C** and **D** as available sources of generating Compounds **A**, **B**, and **E**, we predicted that the level of any of these impurities would eventually reach a plateau and remain below a controlled level and stability data presented in Tables 5 and 6 fully supported the prediction. The increase in the total impurity level by ∼0.2% is thought to be caused by the differences in the UV response factors of various impurities rather than decomposition of the MK-0364 main component. For more accurate quantitation, the individual impurity should be isolated and purified for UV response factor determination when possible. Approximately at 6 months under accelerated conditions and 12 months under long-term conditions, the degradation of the unstable impurities had come to an end as most of Compounds **C** and **D** were consumed.

## 4. Conclusions

Multiple impurities were observed in the MK-0364 drug substance prepared via cyanuric chloride-mediated coupling reaction. These impurities were fully characterized employing reversed phase HPLC coupled with UV or ESI MS detectors. Results of structural elucidation indicated that impurities were formed as various cyanuric chloride derivatives of the chiral amine **1** side chain. Owing to the reactivity of the cyanuric chloride moiety, some impurities are prone to further hydrolysis in solid state or thermal degradation at room temperature, causing significant changes in the impurity profile of the stability samples. Impurity identification and forced degradation studies provided critical information for the degradation pathways which suggested that the root cause of the changes in HPLC impurity profile was the degradation of unstable impurities to more stable compounds. MK-0364 main component remained stable during the entire stability studies. The temperature and humidity can impact the rate of degradation. The higher the temperature and humidity, the faster the degradation occurred. However, the degradation was more rapid in the beginning but reached a plateau after 12 months at 25 °C/60% RH as the source impurities were consumed. Our initial assessment of the impurity stability was eventually supported by the complete stability data set.

## References

[1] L.S. Lin, T.J. Lanza Jr., J.P. Jewell, P. Liu, S.K. Shah, H. Qi, X. Tong, J. Wang, S.S. Xu, T.M. Fong, C.-P. Shen, J. Lao, J.C. Xiao, L.P. Shearman, D.S. Stribling, K. Rosko, A. Strack, D.J. Marsh, Y. Feng, S. Kumar, K. Samuel, W. Yin, L.H.T. Van der Ploeg, M.T. Goulet, W.K. Hagmann, J. Med. Chem. 49 (2006) 7584–7587.
[2] M. Schnelle, F. Strasser, Cannabis and Cannabinoids, Haworth Integrative Healing Press, New York, 2002, pp. 153–164.
[3] A. Drewnowski, J.A. Grinker, Pharmacol. Biochem. Behav. 9 (1978) 619–630.
[4] T.M. Fong, X.-M. Guan, D.J. Marsh, C.-P. Shen, D.S. Stribling, K.M. Rosko, J. Lao, H. Yu, Y. Feng, J.C. Xiao, L.H.T. Van der Ploeg, M.T. Goulet, W.K. Hagmann, L.S. Lin, T.J. Lanza Jr., J.P. Jewell, P. Liu, S.K. Shah, H. Qi, X. Tong, J. Wang, Suoyu S. Xu, B. Francis, A.M. Strack, D.E. MacIntyre, L.P. Shearman, J. Pharm. Exp. Ther. 321 (2007) 1013–1022.
[5] C.-y. Chen, L.F. Frey, S. Shultz, D.J. Wallace, K. Marcantonio, J.F. Payack, E. Vazquez, S.A. Springfield, G. Zhou, P. Liu, G.R. Kieczykowski, A.M. Chen, B.D. Phenix, U. Singh, J. Strine, B. Izzo, S.W. Krska, Org. Process Res. Dev. 11 (2007) 616–623.
[6] K.P. Haval, Synlett 13 (2006) 2156–2157.
[7] European Medicines Agency (EMEA), ICH Topic Q3A (R1), Impurities in New Drug Substances, August 2002.
[8] ICH Harmonised Tripartite Guideline, Q1A (R2), Stability Testing of New Drug Substances and Products, February 2003.
[9] T.W. Bentley, G. Llewellyn, J.A. McAlister, J. Org. Chem. 61 (1996) 7927–7932.
[10] M. Kunishima, Y. Zasshi, Pharm. Soc. Jpn. 128 (2008) 425–438.