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Examination of rofecoxib solution decomposition under alkaline and photolytic stress conditions

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Abstract

Rofecoxib is a highly active and selective *cyclo*-oxygenase II inhibitor. A stability-indicating method for the assay of rofecoxib has been developed using reverse-phase high-performance liquid chromatography (HPLC). Stress testing of rofecoxib was conducted during the method development and validation. HPLC analysis of rofecoxib solutions stressed under alkaline and photolytic conditions revealed the presence of several degradates. Two main degradates were determined to be the cyclization product formed by photo-cyclization and the dicarboxylate formed by ring opening in the presence of base and oxygen. The identities of these degradates were confirmed by comparison of UV spectra and HPLC retention time with the independently synthesized products. The mechanistic pathways for the formation of these degradates are discussed. Further improvement of the HPLC method's ruggedness has been made based on these studies.

Keywords: Rofecoxib; Base-decomposition; Photodecomposition; Stability

1. Introduction

The *cyclo*-oxygenase activity is the site of action of non-steroidal anti-inflammatory drugs (NSAIDS), including aspirin, ibuprofen and indomethacin, which are widely used as anti-inflammatory, analgesic and anti-pyretic agents [1–3]. However, major side effects such as gastritis, ulceration and haemorrhagia are associated with the chronic use of NSAIDS [4]. In the early 1990s it became clear that there are two distinct *cyclo*-oxygenase enzymes: *cyclo*-oxygenase I (COX-1) and *cyclo*-oxygenase II (COX-2). Cox-1 is expressed in normal tissues in the stomach, intestines and kidneys [5–7]. In contrast, COX-2 is found in elevated levels in inflammatory exudate [8]. This discovery lead to the development of rofecoxib [9], a specific COX-2 inhibitor. It selectively targets the prostaglandins involved in pain and inflammation. These benefits are important in the treatment of osteoarthritis, acute pain and primary dysmenorrhea.

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A highly selective reversed-phase high-performance liquid chromatography (HPLC) method was developed to separate impurities and degradates from the rofecoxib drug substance in order to monitor its quality and establish a stability profile. Stress testing of the drug substance, which is carried out under extreme thermal, humid, oxidative, acidic, alkaline, and photolytic conditions, helps to determine the intrinsic stability of the molecule. By establishing degradation pathways, one can identify the potential degradation products and use them to validate the stability-indicating power of the HPLC method. Two major degradates were observed during the stress testing of rofecoxib. The first degradation reaction observed is the base-promoted hydrolysis of the lactone moiety followed by oxidation, which yields the dicarboxylate. The other degradation reaction is a photo-cyclization of the *cis*-stilbene moiety, which yields a phenanthrene derivative. This paper describes the use of reversed-phase HPLC with photo diode array detection (PDA) for the identification of these degradates and elucidation of the postulated mechanistic pathway for their formation. Information obtained from the stress studies proved to be helpful in the development of the HPLC method.

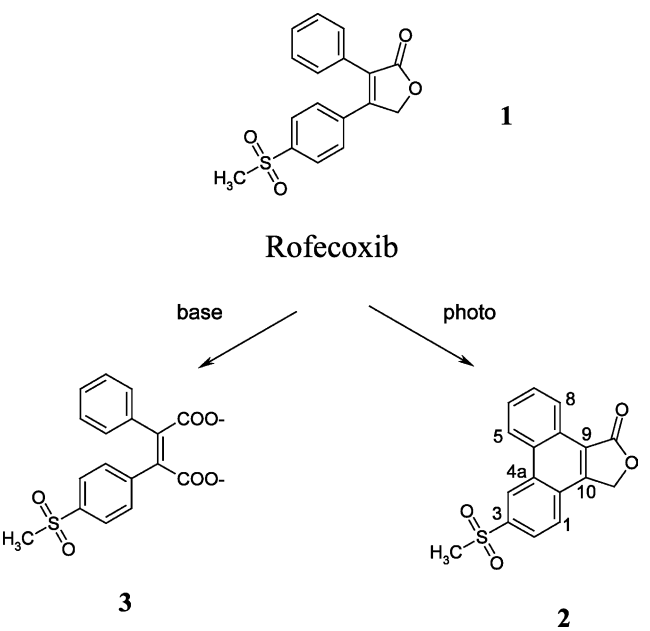
2. Experiment

2.1. Materials

HPLC grade acetonitrile and water and reagent grade H_3PO_4 and NaOH are purchased from Fisher Scientific (Fair Lawn, NJ, USA). Rofecoxib was obtained from Merck Research Laboratories (Rahway, NJ, USA). Rofecoxib degradates were synthesized by the Process Research Department of Merck Research Laboratories (Rahway, NJ, USA).

2.2. Sample preparation

The standard solution for rofecoxib was prepared by weighing 20.0 mg of rofecoxib, transferring into a 100 ml low actinic volumetric flask and diluting to volume with 50:50 H_2O–acetonitrile (concentration 0.2 mg/ml).



Scheme 1.

Table 1
Retention time of photo and base degradates of rofecoxib

		Retention time (min)	Relative retention time	Area%[a]
Rofecoxib		13.4	1.00	–
Photodegradate	**2**	14.5	1.08	1.9
Base degradates	**3**, Peak 1	8.3	0.62	17.4
	5, Peak 2	11.6	0.87	4.5
	4, Peak 3	17.1	1.28	9.5

[a] Area% of photodegradation for 1 h and of base degradation for 30 min.

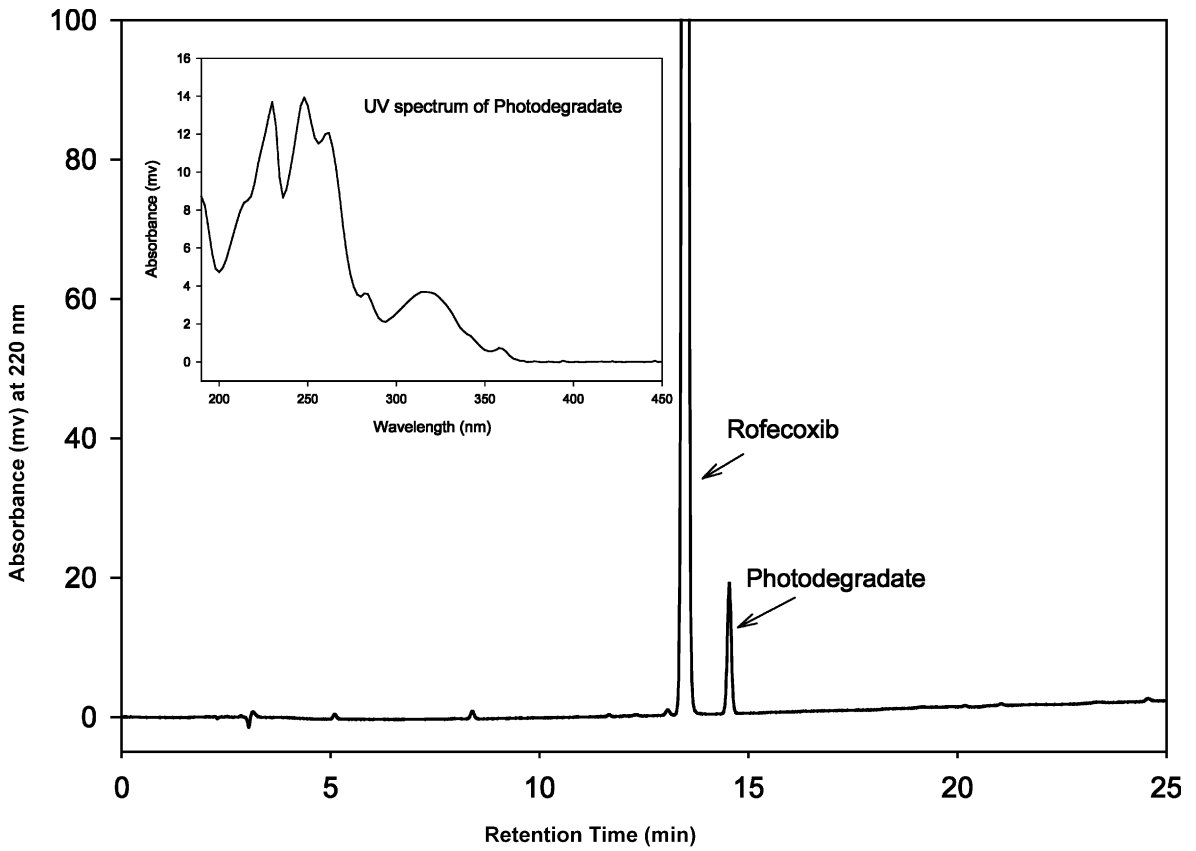


Fig. 1. Chromatogram of a photo stressed rofecoxib solution. Insert: ultraviolet spectrum of the photodegradate eluting at 14.5 min in the HPLC system. The chromatographic conditions are described in Section 2.4.

2.3. Preparation of rofecoxib degradation solutions

2.3.1. Base degradation solution

A 200 μl volume of 1.0 N NaOH was added to 100 ml rofecoxib solution at 0.2 mg/ml concentration in 50:50 H_2O–acetonitrile and the resulting solution held for 30 min, then phosphoric acid was added to the solution at 0.1 v/v% concentration to quench the reaction. A 10 μl volume was injected into the HPLC system.

2.3.2. Photodegradation solution

A rofecoxib solution at 0.2 mg/ml concentration in 50:50 H_2O–acetonitrile was placed under room light in a clear volumetric for 1 h. A 10 μl volume was injected into the HPLC system.

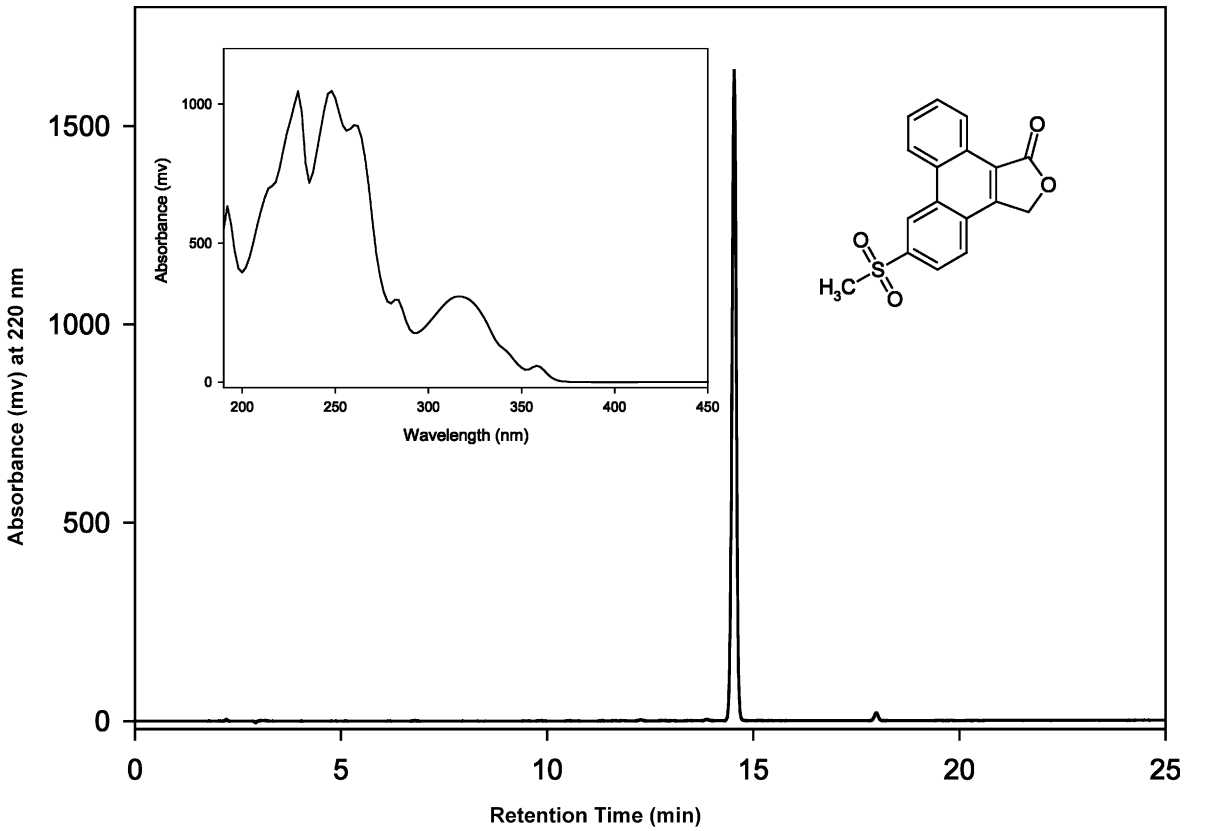
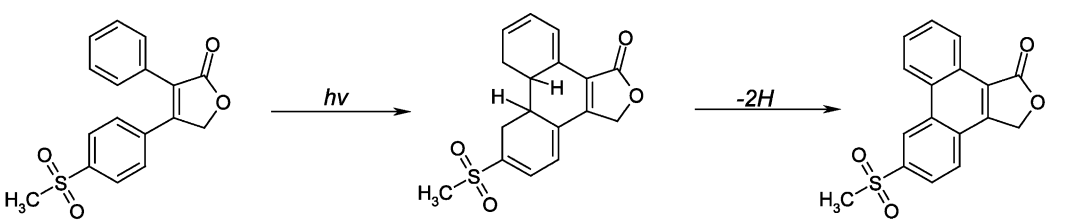


Fig. 2. Chromatogram of the independently synthesized phenanthrene derivative and its ultraviolet spectrum in the insert. The chromatographic conditions are described in Section 2.4.



Scheme 2.

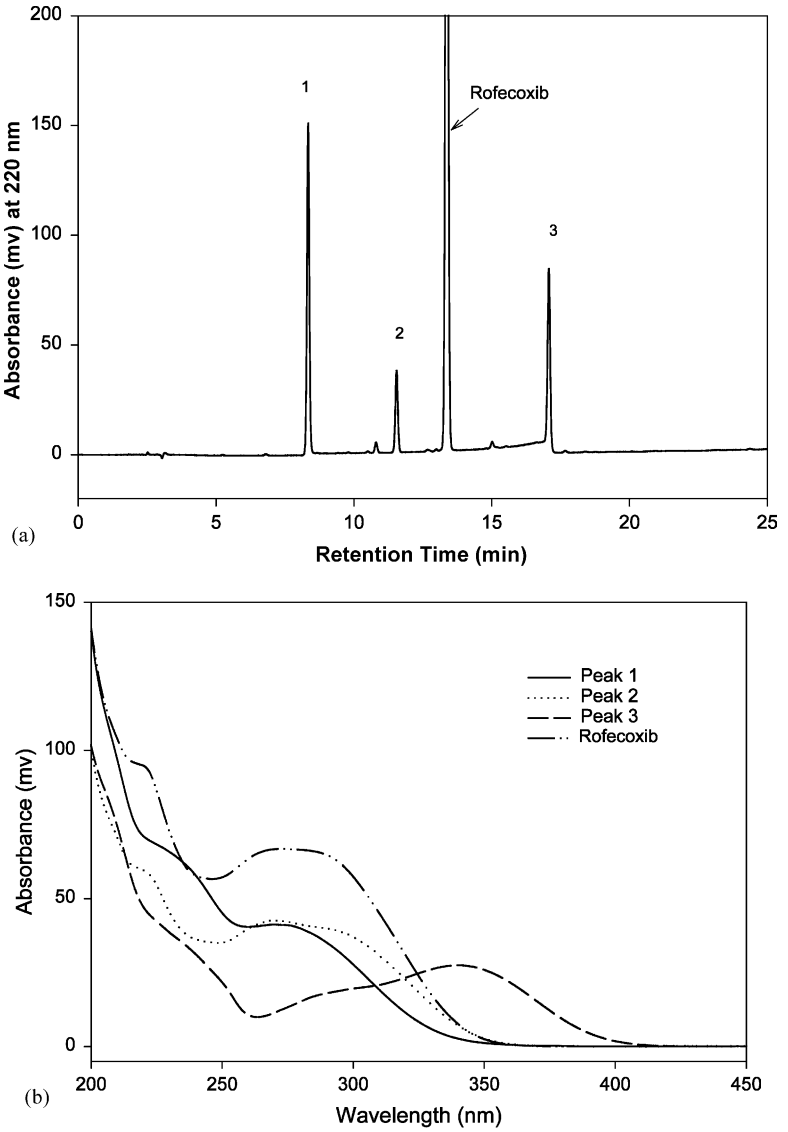


Fig. 3. (A) Chromatogram of a base stressed rofecoxib solution. (B) Ultraviolet spectrum of rofecoxib and the three degradates eluting at 8.3, 11.6 and 17.1 min in the HPLC system. The chromatographic conditions are described in Section 2.4.

2.4. HPLC conditions

The HPLC system consisted of a Hewlett–Packard model 1100 liquid chromatograph that included an auto-injector, a binary pump, and a photo-diode-array detector. Test solutions were automatically injected onto a 25 cm × 4.6 mm I.D. (5 μm particle) Waters Symmetry C8 column (Waters). The mobile phase of 0.1% H_3PO_4 (Component A) and acetonitrile (Component B) at a flow rate of 1.0 ml/min, employs a linear gradient from 70:30 A–B (v/v) to 40:60 A–B (v/v) over 15 min, then to 15:85 A–B (v/v) over 10 min. Detection was by UV at 220 nm and PDA.

2.5. NMR

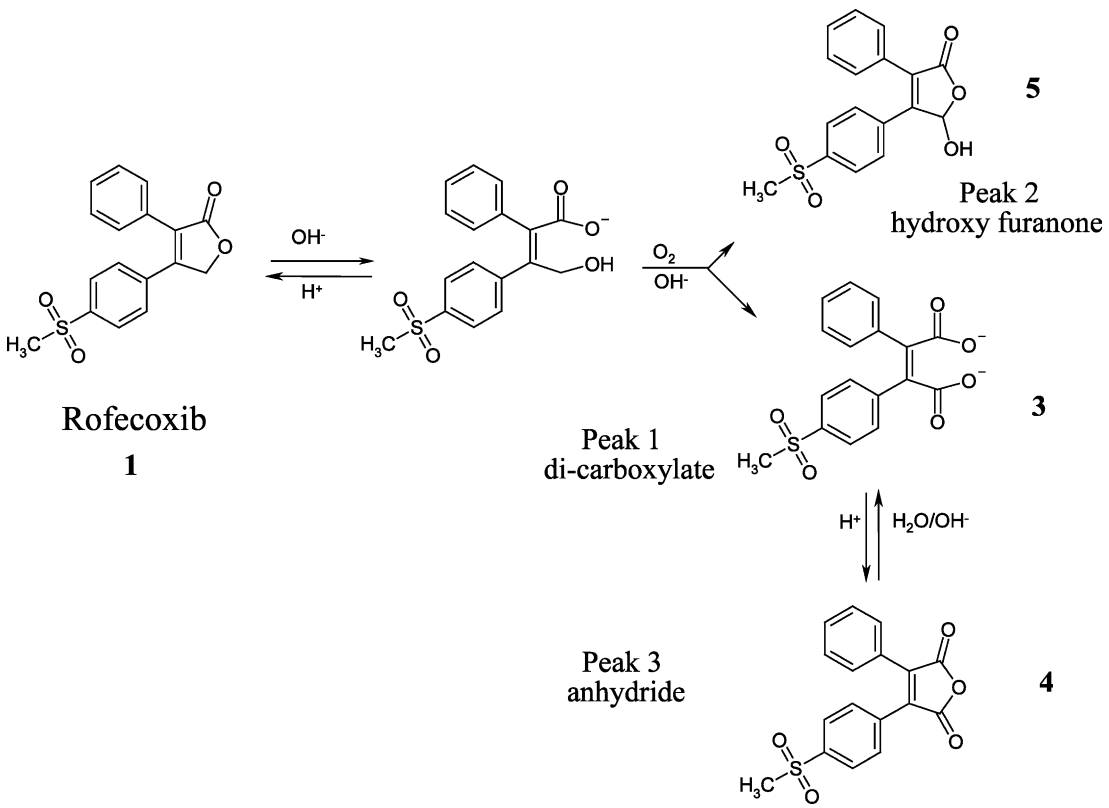
Proton and ^{13}C NMR studies were performed in Bruker AMX-400 and Avance-500 NMR spectrometers. Proton spectra were referenced to DMSO-d_5 ($\delta = 2.50$ ppm) and ^{13}C spectra were referenced to DMSO-d_6 ($\delta = 39.5$ ppm). Two-dimensional $^{1}H-^{13}C$ long-range correlation experiments (HMBC) were used to characterize all compounds.

3. Results and discussion

To further evaluate the selectivity of the HPLC method for analysis of rofecoxib drug substance, degradation products were purposely generated through exposure of the rofecoxib **1** (Scheme 1) to extreme thermal, humidity, oxidative, acidic, alkaline, and photolytic conditions. The main degradates of rofecoxib are formed by base-catalyzed hydrolysis and photo induced cyclization (Table 1). The major degradate produced in alkaline solution is the ring-opened dicarboxylate and the major photodegradate is a phenanthrene derivative, which is formed by photo-induced cyclization (Scheme 1).

3.1. Identification of photodegradate

A typical chromatogram of rofecoxib solution after exposure to light as described in Section 2 is shown in Fig. 1. One major photodegradate was detected, eluting at 14.5 min in the HPLC system described in Section 2. The structure of this photodegradate was established as a substituted phenanthrene **2** (Scheme 1) by NMR analysis of the isolated degradate from a photo stressed rofecoxib solution. Gradient nuclear overhauser effect (NOE) studies [10,11] were used to verify the



Scheme 3.

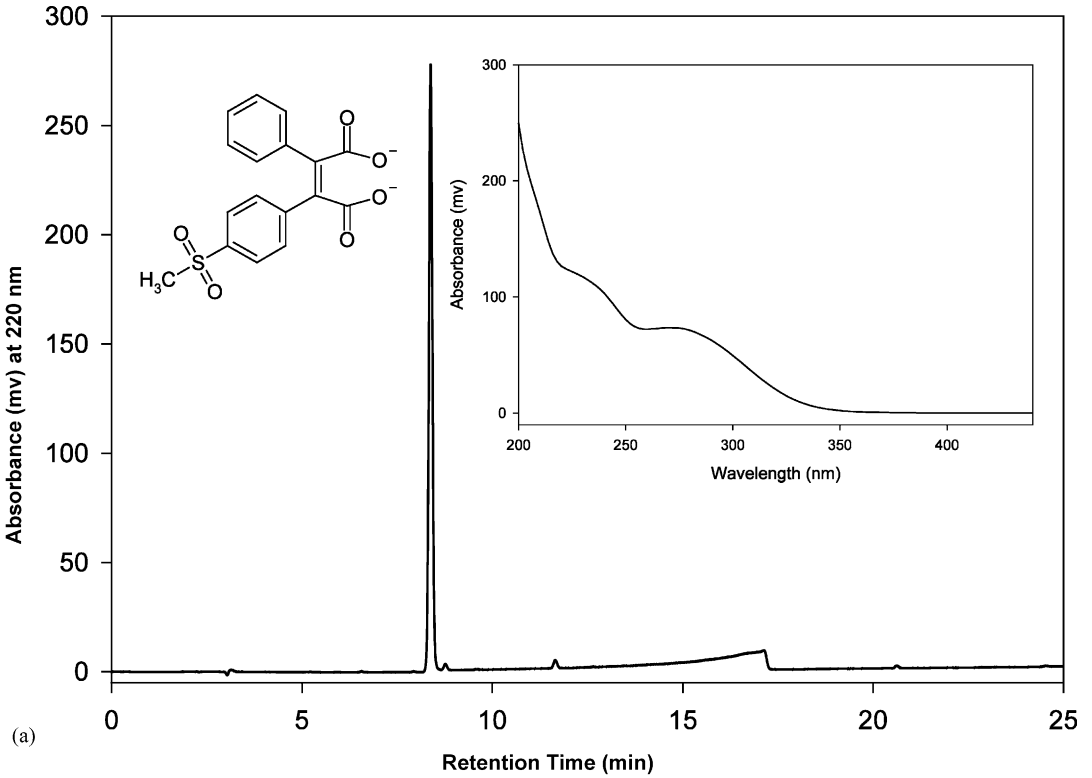


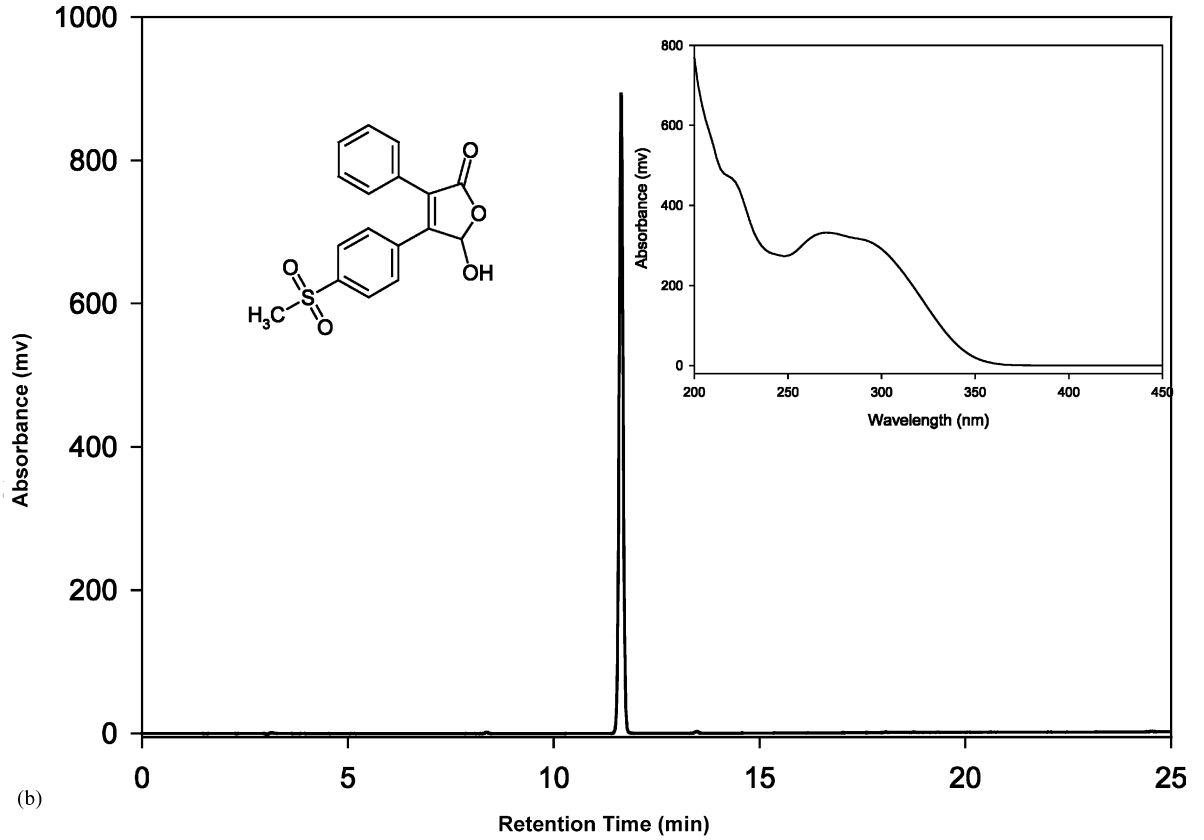
Fig. 4. (A) Chromatogram of the independently synthesized dicarboxylate derivative of rofecoxib and its ultraviolet spectrum in the insert. (B) Chromatogram of the synthesized hydroxyfuranone derivative of rofecoxib and its ultraviolet spectrum in the insert. (C) Chromatogram of the synthesized anhydride derivative of rofecoxib and its ultraviolet spectrum in the insert. The chromatographic conditions are described in Section 2.4.

structure of **2**. Key NOE enhancements were observed from OCH_2 ($\delta = 5.85$ ppm) to H_1 ($\delta =$ 8.34 ppm) and from H_4 ($\delta =$ 9.42) to H_5 ($\delta =$ 9.04). Additionally, the low field chemical shifts of these aromatic ring protons are consistent with a phenanthrene ring system. The identification was further confirmed by the comparison of the diode-array UV spectrum of the rofecoxib photodegradate with the spectrum of the synthesized product as well as by their matching retention times (Fig. 2). The identification of the photodegradate was also aided by the structural comparison of *cis*-stilbene (*cis*-diarylethylenes) and rofecoxib. Rofecoxib can be considered a substituted *cis*-stilbene. The photoreactions of *cis*-stilbene have been studied extensively as models of photo isomerization and photocyclization [12]. *cis*-Stilbene undergoes *cis–trans* photoisomerization, as well as a photochemical ring closure reaction to form 4a,4b-dihydrophenanthrene which dehydrogenates in the presence of oxygen to form phenanthrene [13,14]. A possible pathway for photocyclization of rofecoxib is shown in Scheme 2. The intermediate 4a,4b-dihydrophenanthrene product was not observed, however, it has been established that the majority of the 4a,4b-dihydrophenantherenes are unstable at room temperature or below and undergo rapid dehydrogenation by oxygen, especially for the substituted diaryl ethylenes like rofecoxib [13,14]. It should be noted that the photo degradation of rofecoxib discussed here occurred in a solution. Rofecoxib is stable in the solid state under the ICH light exposure conditions, with only minor degradation (0.05 A%) observed under UV light.

3.2. Identification of base degradates

A typical chromatogram of base-stressed rofecoxib solution as described in Section 2 is shown in Fig. 3A. Three major degradates can be easily observed at 8.3, 11.6 and 17.1 min (Table 1). The UV spectra of these degradates and the UV spectrum of rofecoxib are shown in Fig. 3B. The identities of the three degradates were determined to be a ring opened dicarboxylate **3**, a hydroxyfuranone **5**, and an anhydride of rofecoxib **4** (Scheme 3), respectively. The structures of all three were established through the NMR analyses [15] of samples prepared by independent synthesis. The conclusion was confirmed further by the matching UV spectrum and retention time (Fig. 4A–C) of each compound prepared by independent synthesis.

The effect of oxygen on the base degradation reaction was also investigated. A 200 μl volume of 1.0 N NaOH was added to 100 ml rofecoxib solution at 0.2 mg/ml concentration in 50:50 H_2O–acetonitrile, which was being flushed with helium. The reaction was allowed to proceed for 30 min with continued helium flush, then quenched by the addition of phosphoric acid to 0.1 v/v% concentration. A second rofecoxib solution was stressed with base under exactly the same conditions except without helium flushing. The chromatograms are shown in Fig. 5. Three degradation products were observed in the system that was without helium flushing (Fig. 5A), however, we did not observe any significant degradates in the system with helium flushing (Fig. 5B). This result established that an additional oxidation step is involved in the reaction. In a separate



(b)

Fig. 4. (*Continued*)

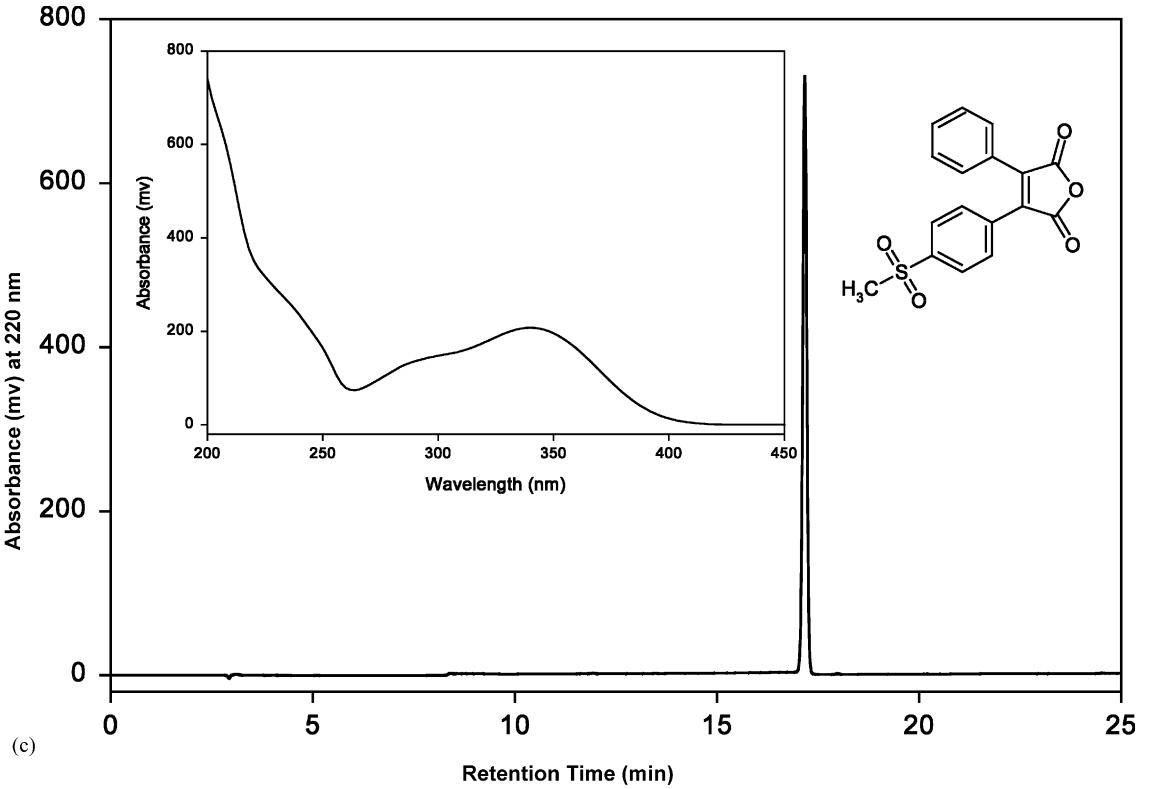


Fig. 4. (*Continued*)

oxidative stress study, a rofecoxib solution containing 6% hydrogen peroxide was held for 3 h and no significant oxidative degradates were observed (data not shown). These studies indicate that the base degradation is a combination of hydrolysis followed by oxidation.

The most plausible pathway for the alkaline hydrolysis of rofecoxib is shown in Scheme 3. Rofecoxib has a γ-lactone ring moiety. Base-promoted hydrolysis of lactone or ester is well established [15]. The opening of the lactone ring is catalyzed by hydroxide ion via nucleophilic addition at the carbonyl carbon, which produces ring-opened carboxylate with a hydroxyl group. If the pH of the reaction medium is lowered, then lactonization or cyclization is expected to occur. The kinetic and mechanistic studies of the alkaline hydrolysis for several alkaloids that contain lactone rings have been reported in the literature. Camptothecin **6** (Scheme 4), a plant alkaloid found to be active against L1210 leukemia [16], contains a terminal α-hydroxy-δ-lactone ring. The lactone ring is labile and is easily hydrolyzed to form sodium camptothecin in alkaline medium [17] and the reaction is reversible in acidic medium. Sercurinine **7** (Scheme 4), a major alkaloid from the genus *Securinega* reported to be useful in the treatment of paralysis and physical disorders [18], contains a α-vinyl-γ-lactone ring. Sercurinine undergoes ring opening lactone hydrolysis at high pH, and the hydrolyzed lactone ring cyclizes rapidly to yield sercurinine under acidic pH [18]. Similar to camptothecin and sercurinine, our results indicate that the exchanging between the ring opened carboxylate form and the ring closed lactone form exists in rofecoxib by varying the solution pH in the absence of oxygen (Fig. 5B). Although there is no direct evidence showing the existence of the ring opening carboxylate of rofecoxib, the fact that oxidation can-

not occur in solution independent of the base promoted hydrolysis does indicate indirectly the presence of the ring opened hydrolysis product. However, unlike camptothecin and sercurinine, the ring opening hydrolysis product of rofecoxib is unstable. It either undergoes a cyclization reaction producing rofecoxib in an acidic medium (Fig. 5B) or an oxidation reaction in the presence of molecular oxygen in an alkaline medium (Fig. 5A). The most likely oxidation pathway is the oxidation of the γ-primary alcohol of the hydrolyzed rofecoxib. Primary alcohols can be oxidized to aldehydes and carboxylic acids, and easily beyond the aldehyde stage in aqueous solutions [19]. Presumably, hydroxyfuranone **5** (Scheme 3) can be formed through cyclization in the first stage oxidation, and further oxidation will lead to the formation of carboxylic acid **3**

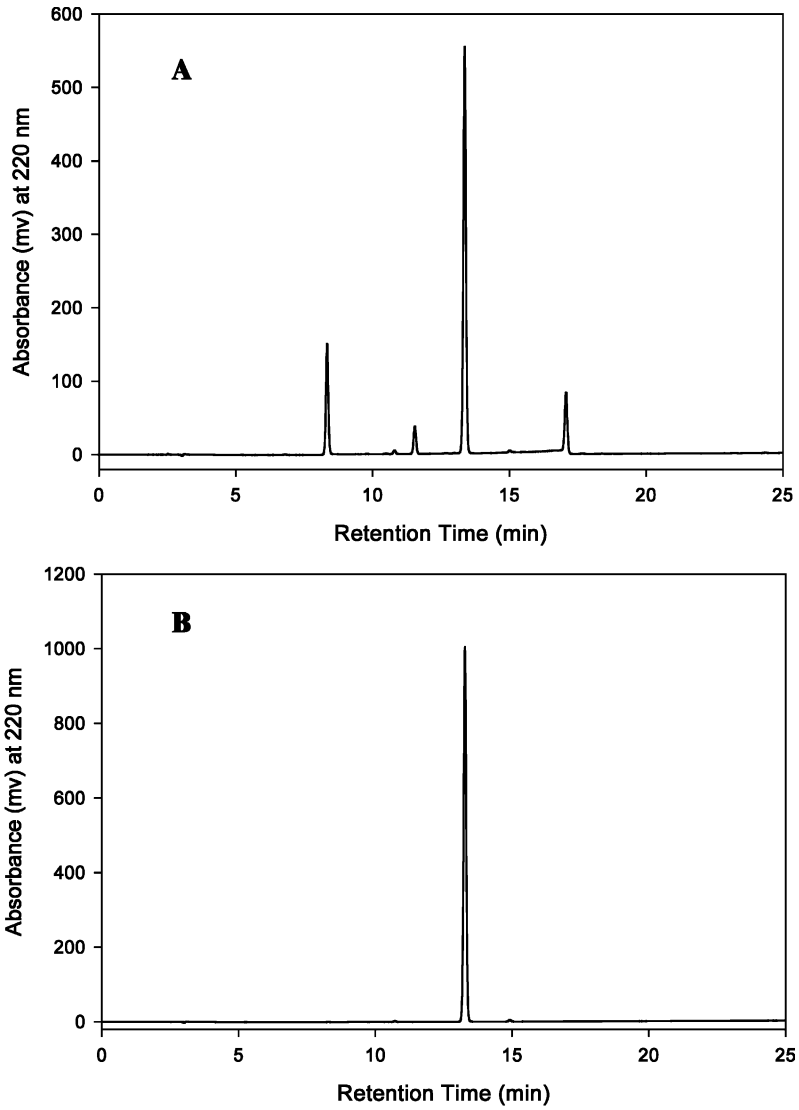
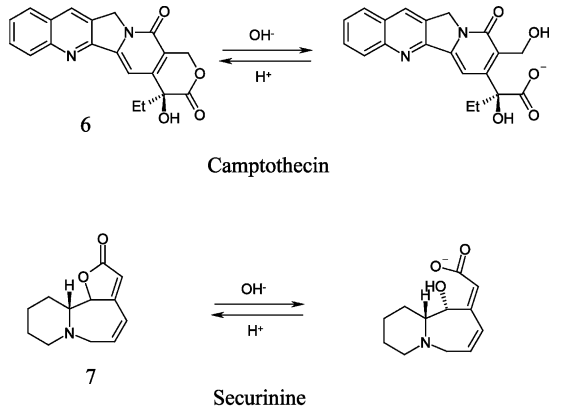


Fig. 5. Chromatograms of base stressed rofecoxib solutions (A) without helium flush and (B) with helium flush. The chromatographic conditions are described in Section 2.4.



Scheme 4.

(Scheme 3). The present data are not sufficient to confirm these pathways, however, the presence of hydroxyfuranone degradate provides evidence for this hypothesis. Meanwhile, the relatively low yield of the hydroxyfuranone **5** in comparison with the dicarboxylate **3** and the anhydride **4** (Table 1) indicates that the preferred pathway from the primary alcohol is a continued two step oxidation to dicarboxylate under these conditions.

The dicarboxylate **3** and the anhydride form **4** can be inter-converted easily by varying the solution pH as shown in Scheme 3. To illustrate the conversion, the synthesized dicarboxylate and anhydride solutions were prepared using different diluents. The inter-conversions can be easily observed in the impurity profile as shown in Fig. 6. The dicarboxylate solution prepared under neutral conditions (50:50 water–acetonitrile) does not exhibit significant anhydride form (Fig. 6A), however, under acidic conditions (dissolved in 50:50 water–acetonitrile in 0.1v/v% H_3PO_4) significant amounts of dicarboxylate are converted into the anhydride form (Fig. 6B). On the other hand, when dissolved in acetonitrile, the anhydride form is rather stable (Fig. 6C), however, in the presence of water (50:50 water–acetonitrile) it is easily hydrolyzed (Fig. 6D). These results help to explain the broad shoulder of the anhydride (peak 3 in Fig. 3) in the base stressed rofecoxib. Since one of the mobile phases used in the HPLC method is 0.1% H_3PO_4 in H_2O, the broad shoulder is the result of on-column conversion of the dicarboxylate form to the anhydride form under the acidic conditions. The results also suggest that the anhy-

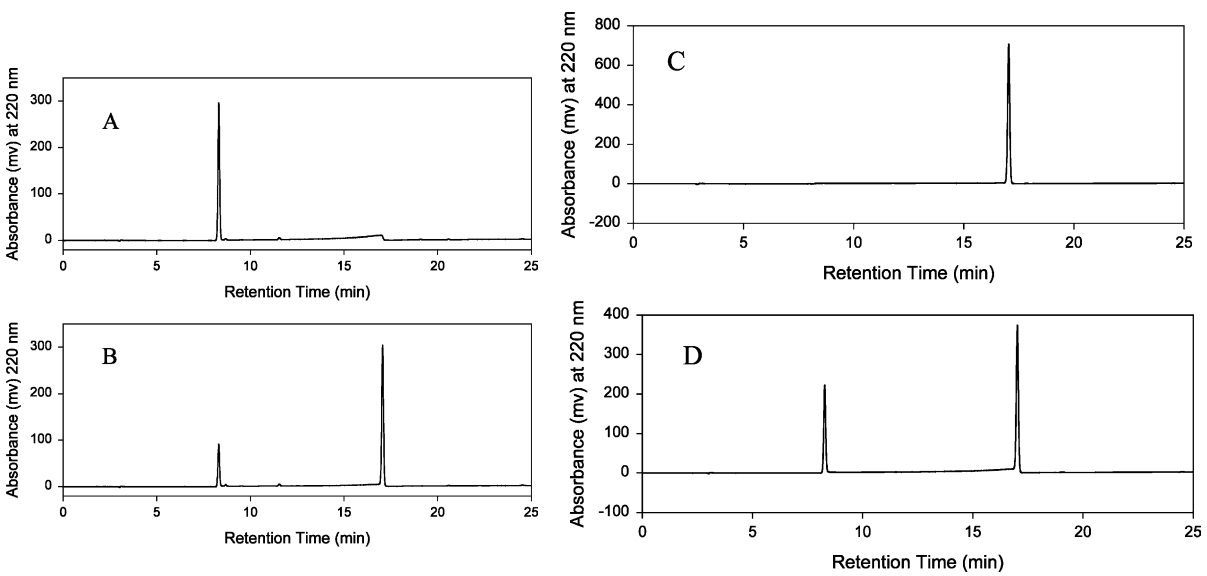


Fig. 6. (A) Chromatogram of rofecoxib dicarboxylate in 50:50 water–acetonitrile solution. (B) Chromatogram of rofecoxib dicarboxylate in 50:50 water–acetonitrile 0.1% v/v phosphoric acid solution. (C) Chromatogram of rofecoxib anhydride in acetonitrile solution. (D) Chromatogram of rofecoxib anhydride in 50:50 water–acetonitrile solution. The chromatographic conditions are described in Section 2.4.

dride form **4** (Scheme 3) observed in the base degradation of rofecoxib (Fig. 3) is the result of the acid quench at end of reaction which converts some of the dicarboxylate product to its anhydride form. The main base degradate is the ring opened dicarboxylate form **3**.

3.3. Improvement of the HPLC method's ruggedness

The impurity profile method was developed to monitor the quality of drug substance (as described in Section 2). In the early development stage, 50:50 H_2O and acetonitrile were used as the diluent. A low level (0.02–0.09% by area) unknown impurity at relative retention time (RRT) = 0.62 has infrequently been observed in the impurity profile. The occurrence and the level of this impurity varied with different lots of vials that were used and how long the sample solution had been kept in the vial. This suggested that this impurity is not related to the quality of the drug substance, rather it is most likely a degradation product.

The UV spectrum and the retention time comparison confirmed that the observed impurity is dicarboxylate **3**. The formation of this impurity is most likely catalyzed by trace amounts of base on the vial surface. Consequently, this impurity was eliminated and the method's ruggedness was improved by using acidified diluent (0.1% H_3PO_4 in 50:50 water–acetonitrile) to prevent the alkaline hydrolysis.

As a result of the studies described above, sample solutions of rofecoxib are prepared in the dark with low actinic volumetrics and dissolved in acidified diluent during routine analysis.

4. Conclusions

From this study it can be concluded that rofecoxib is a base-sensitive drug, its stability can be affected by base catalyzed hydrolysis in solution followed by oxidation to form a major ring opened dicarboxylate degradate and minor hydroxyfuranone degradate of rofecoxib. Rofecoxib solution is photosensitive; photo cyclization of rofecoxib in solution produces a phenanthrene derivative. Based on the information from the stress testing, the HPLC method's ruggedness was further improved by preparing sample solutions in the dark to avoid photodecomposition and by using acidified diluent to eliminate base degradation. The mechanistic pathways for both degradations are proposed. Further studies on the kinetics of both degradations will be conducted to corroborate these reaction pathways.

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[11] NMR data for **2** (photodegradate) ^{1}H NMR (500.13 MHz, DMSO-d_6) δ 9.42 (d, $J = 1.6$ Hz, 1H), 9.04 (m, 1H), 8.94 (m, 1H), 8.34 (d, $J = 8.3$ Hz, 1H), 8.28 (dd, $J = 8.3$ Hz, 1.6, 1H), 7.90 (m, 2H), 5.85 (s, 2H), 3.46 (s,

3H); ^{13}C NMR (125.76 MHz, DMSO-d_6) δ 170.9, 149.1, 141.9, 132.0, 129.9, 129.2, 128.5, 127.5, 126.9, 126.2, 124.6, 124.2, 123.3, 123.1, 120.1, 68.9, 43.3.

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[15] NMR data for **3** (dicarboxylate as bisdiisopropylamine salt) ^{1}H NMR (399.87 MHz, DMSO-d_6) δ 8.4 (v br, 4H), 7.57 (m, 2H), 7.20 (m, 2H), 7.05 (m, 2H), 7.02 (m, 1H), 6.97 (m, 2H), 3.11 (septet, $J = 6.4$ Hz, 4H), 3.09 (s, 3H), 1.10 (d, $J = 6.4$ Hz, 24H); ^{13}C NMR (100.55 MHz, DMSO-d_6) δ 169.1, 168.6, 145.9, 142.8, 139.9, 139.4, 137.8, 130.4, 129.3, 127.0, 126.0, 125.4, 45.4, 43.5, 20.4; **4** (anhydride) ^{1}H NMR (399.87 MHz, DMSO-d_6) δ 8.03 (d, $J = 8.4$ Hz, 2H), 7.68 (d, $J = 8.4$ Hz, 2H), 7.55–7.43 (om, 5H), 3.28 (s, 3H); ^{13}C NMR (100.55 MHz, DMSO-d_6) δ 164.8, 164.6, 142.1, 140.1, 136.9, 132.6, 131.0, 130.3, 129.5, 128.9, 127.3, 126.9, 43.2; **5** (hydroxyfuranone) ^{1}H NMR (400.13 MHz, DMSO-d_6) δ 8.05 (s, 1H), 7.95 (m, 2H), 7.62 (m, 2H), 7.44–7.40 (om, 3H), 7.33 (m, 2H), 6.71 (s, 1H), 3.25 (s, 3H); ^{13}C NMR (100.61 MHz, DMSO-d_6) δ 169.8, 154.8, 141.6, 135.8, 129.4, 129.2$_2$, 129.1$_7$, 129.1, 128.7, 127.2, 97.5, 43.1.

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