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Peak fronting in reversed-phase high-performance liquid chromatography: a study of the chromatographic behavior of oxycodone hydrochloride

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Abstract

Severe peak asymmetry —fronting— was observed for oxycodone during elution at 30°C from a C_{18} HPLC column using a mobile phase consisting of 14.9% MeOH, 84.5% 0.05 M KH_2PO_4 (pH 3.0), 0.5% MTBE, and 0.1% TEA. Investigation using deuterium-labeled oxycodone and analysis by LC/MS showed that gem–diol and hemiketal adducts of oxycodone formed as a result of the equilibrium addition of water and methanol to the C-6 ketone on oxycodone. As a result of slow equilibrium kinetics at room temperature in aqueous solution, the gem–diol and methyl hemiketal eluted as an unresolved broad band in front of the oxycodone peak. Decreasing the column temperature to 0°C decreased the rates of interconversion and allowed the resolution and separation of these species from each other and from oxycodone. Increasing the column temperature to 60°C increased the rates of interconversion with the result that the three species eluted as a single, homogenous peak with greatly improved peak symmetry.(Abstract is approximately 157 words)

Keywords: Peak fronting; Oxycodone hydrochloride; Reversed-phase high-performance liquid chromatography

1. Introduction

The management of debilitating pain remains one of the pressing challenges in medical science. Despite tremendous advances over the last few decades, use of opiates continues to be one of the few effective treatments. Sustained-release formulations of morphine and now, oxycodone, provide the physician with effective tools for the management of debilitating pain. OxyContin™ tablets, a unique formulation of oxycodone hydrochloride that utilizes the patented AcroContin™ delivery system [1], allows convenient oral-dose administration every 12 h.

At the time we began work on this project, the United States Pharmacopeia XXII specified the use of one ion-pairing mobile phase for the assay and another for the determination of related compounds for the reversed-phase chromatographic analysis of the therapeutically important opiate alkaloid, oxycodone HCl (I, Fig. 1). However, the ion-pairing reagents and high pH mobile phase

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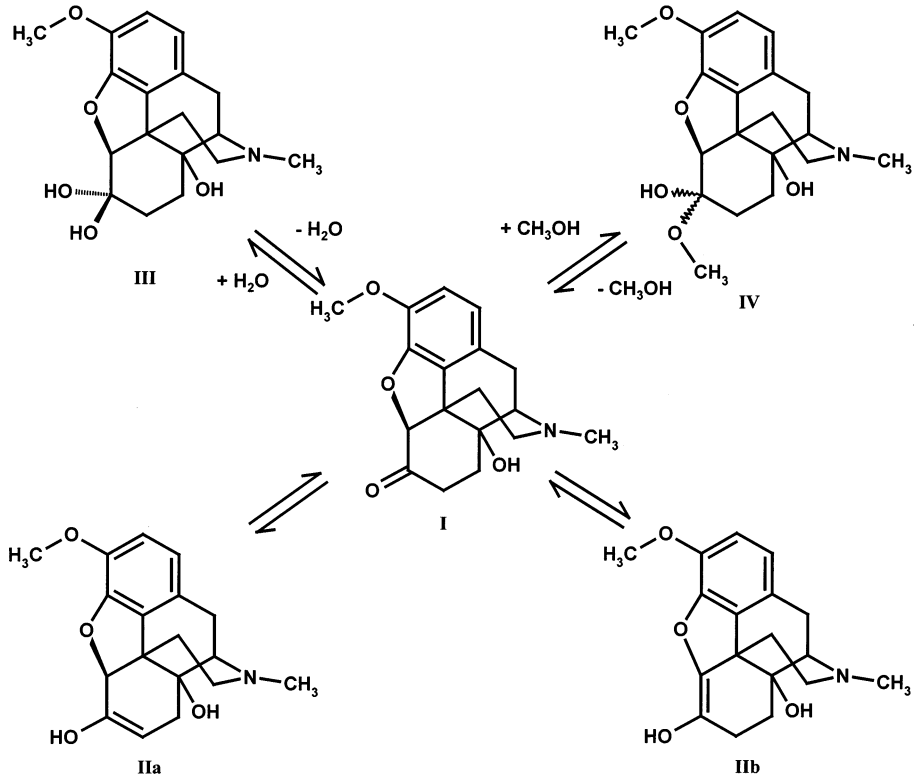


Fig. 1. Structures and equilibria of oxycodone (I) and its acid-catalyzed keto–enol (IIa and b), ketone–gemdiol (III), and ketone–hemiketal (IV) equilbria species.

used in these methods caused rapid deterioration of silica-based reversed-phase column media. To overcome these shortcomings, we developed a new stability-indicating method for the analysis of oxycodone HCl raw material and oxycodone HCl–containing drug products that eliminated the use of ion-pairing reagents and high pH mobile phases.

The new method employs a *low-acidity* HPLC column (μBondapak C_{18}, Waters, Milford, MA) and a mobile phase consisting of 14.9% MeOH, 84.5% 0.05 M KH_2PO_4 (pH 3.0), 0.5% MTBE, and 0.1% TEA. This new chromatographic system exhibits excellent separation efficiency, as measured by the number of theoretical plates, and resulted in the elution of a symmetrical oxycodone peak. It also resolved two previously unknown related compounds. During method development, however, extensive peak *fronting* was observed. This paper reports the results of the investigation into the cause of this peak fronting.

The chromatography of organic bases on silica-based reversed-phase HPLC columns is complicated by adsorption and ion-exchange interactions of the free silanols with amine moieties. These interactions are thought to be the cause of the severe peak tailing and band broadening often observed in the chromatography of amines. Increased separation efficiency and improved peak shapes of organic amines have been observed with less acidic reversed-phase columns, buffers containing potassium rather than sodium salts, increased ionic strength, low pH, and adding amine modifiers such as triethylamine and dimethyloctylamine [2].

Though *peak tailing* has been well discussed in the literature [3–5], there has been little discussion of *peak fronting*. Snyder and Kirkland associate fronting with the 'slow reaction' of the sample during the separation process [6]. An example of

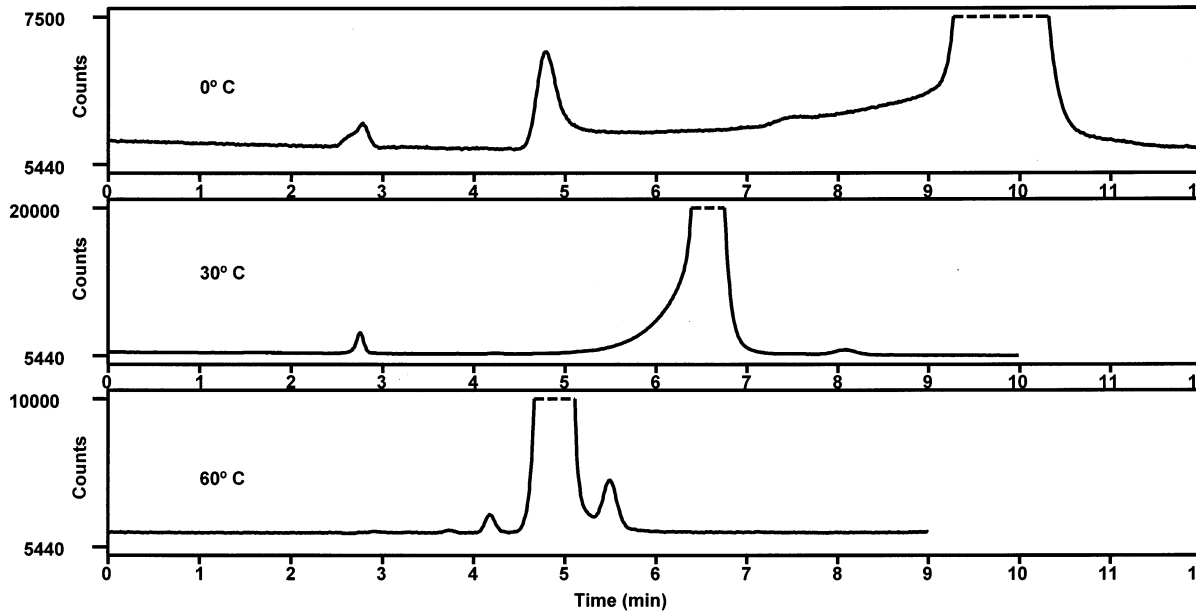


Fig. 2. Chromatograms of oxycodone at 0C, 30C, and 60°C using the chromatographic conditions described in the Experimental section.

peak fronting due to equilibria-related causes involves the interconversion of conformers around the amide bond of captopril during its chromatography on a C_{18} column [7]. In this example, the kinetics of the conformational equilibria were such that when the mutarotation was sufficiently fast relative to the time scale of the chromatographic process, a single symmetrical peak was observed. As the temperature decreased, fronting increased, indicating a slower rate of mutarotation compared to the time scale of the separation. Since oxycodone cannot exhibit conformational equilibria, we investigated other possibilities for the cause of peak fronting of oxycodone.

2. Experimental

2.1. Chemicals

Oxycodone HCl was purchased from Mallinckrodt (St. Louis, MO), methanol, HPLC grade, was purchased from Mallinckrodt (Paris, KY); acetic acid, analytical reagent grade; ammonium hydroxide, ACS reagent grade; hydrochloric acid, ACS reagent grade; and potassium dihydrogen phosphate (KH_2PO_4), ACS reagent grade were purchased from J.T. Baker (Phillipsburg, NJ), sodium hydride, dry; ammonium chloride hexahydrate, 99%; triethylamine 99% (TEA); and methyl *t*-butyl ether (MTBE) were purchased from Aldrich (Milwaukee, WI); methyl alcohol-d, 99.5 + % atom D, was purchased from Janssen (Oshkosh, NJ). HPLC-grade water was generated by the Milli-Q water system (Millipore, Bedford, MA).

2.2. Mobile phases

The mobile phase used for routine chromatography consisted of methanol (v/v 14.9%) and 0.05 M KH_2PO_4 (v/v 84.5%) with 0.1% TEA at pH 3.0 and 0.5% MTBE. The mobile phase used for the LC/MS experiments consisted of methanol (v/v 15%) and water (v/v 85%) acidified to pH 3.0 with acetic acid.

2.3. Instrumentation

The chromatographic system comprised a Hitachi L-6000 isocratic pump, AS-4000 auto-

ampler, and L-4000 UV detector (Hitachi USA, San Jose, CA), a Waters 996 Diode Array Detector (Waters, Milford, MA), and a block column heater with a model MGW Lauda RM6 recirculating water bath (Brinkmann, Westbury, NY). The liquid chromatography/mass spectrometry system comprised a Hewlett–Packard Model 1090M LC equipped with column heater, and photodiode array detector (Hewlett–Packard, Palo Alto, CA) interfaced to a Finnigan MAT SSQ-7000 single quadruple mass spectrometer and Finnigan electrospray ion source (Finnigan-MAT, San Jose, CA). A Waters μBondapak C_{18} (3.9 mm × 300 mm) HPLC column was used in both systems. NMR spectra were obtained in CD_2Cl_2 with a JEOL Eclipse 400 MHz Spectrometer using the Delta Processing and Control software v1.6 (JEOL, Peabody, MA) [8].

2.4. Experimental conditions

For HPLC analysis, samples were dissolved in 0.85% H_3PO_4 at about 0.2 mg ml^{-1}. The HPLC conditions for both routine and LC/MS analysis were: flow rate, 1.0 ml min^{-1}, UV detection, 230 nm, and injection volume, 20 μl; the electrospray needle for the LC/MS experiments was maintained at 4.5 eV.

2.5. Deuteration of oxycodone

A 25 ml two-neck, round bottom flask and Teflon-coated magnetic stir-bar were dried at 105°C, flushed with argon, and charged with 14 ml of CH_3OD. Sodium hydride (3.5 mg) followed by oxycodone (341 mg) were added and stirring of the suspension started. The ratio of the molecular ions due to the parent compound, mono-, di-, and tri-deuterated species was monitored by mass spectrometry over several days. When no ions for the parent compound or mono-deuterated oxycodone could be detected and the ratio of the di- and tri-deuterated species remained constant as determined by mass spectrometry, the reaction was quenched with the addition of 3 ml of acidified NH_4Cl (1 g NH_4Cl/100 ml H_2O, and 1 ml concentrated HCl, ≈ pH 2). The addition of the acidified NH_4Cl caused all solids to dissolve and the reaction mixture to become homogeneous. The solution was made alkaline with 6 M NH_4OH and the resulting precipitate collected by vacuum filtration and dried at 105°C.

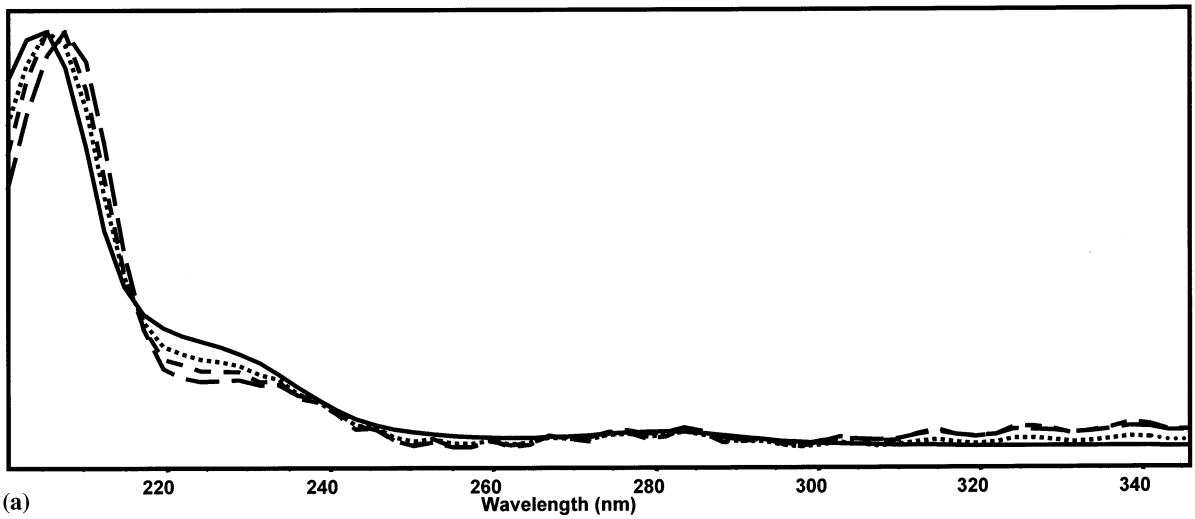
Mass spectral analysis of the isolated product showed it to consist of 88.6% tri-deuterated oxycodone, and 8.6% di-, and 2.8% mono-deuterated oxycodone. The appearance of mono-deuterated oxycodone in the final product was unexpected because it was not detected in the reaction mixture prior to quenching; it was presumably occluded in the suspended solids. NMR analysis confirmed the location and extent of deuteration and showed that the product was 91.5% deuterated at C-7, and 93.2% deuterated at C-5. The NMR data is in excellent agreement with that obtained by mass spectral analysis.

3. Results

3.1. Effect of column temperature on peak shape

The chromatograms obtained at column temperatures of 0, 30, and 60°C are shown in Fig. 2. As can be seen from these chromatograms, increasing the column temperature had a dramatic effect on fronting: As the column temperature increased, the tailing factor, unmeasurable at 0°C, increased from 0.7 at 30°C to 0.95 at 60°C. At a

Fig. 3. For the chromatogram shown in Fig. 2a, column temperature, 0°C; superimposition of photodiode array UV spectra of eluate at retention times of: – – – – – –, 4.8 min; – – – –, 7.5 min; ·····, 8.5 min; and ––––––, 10.0 min. For the chromatogram shown in Fig. 2b, column temperature, 30°C, superimposition of photodiode array UV spectra of eluate at retention times of: – – – – – –, 5.8 min; ·····, 6.2 min; and ––––––, 10.0 min. For the chromatogram shown in Fig. 2c, column temperature, 60°C; superimposition of photodiode array UV spectra of eluate at retention times of: – – – –, 4.7 min; ····· ,4.8 min; and ––––––5.0 min.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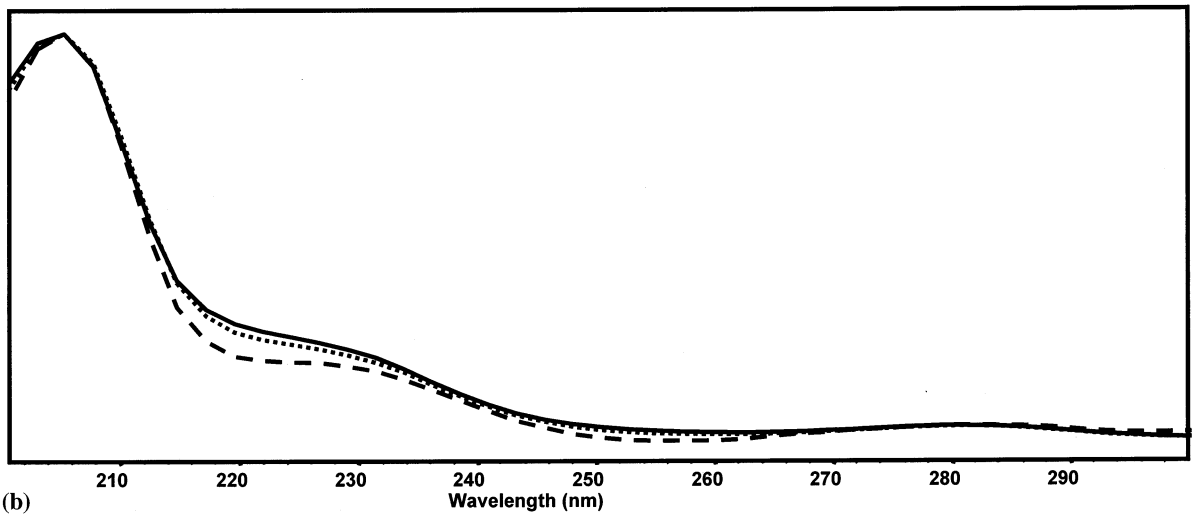
(a)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(b)

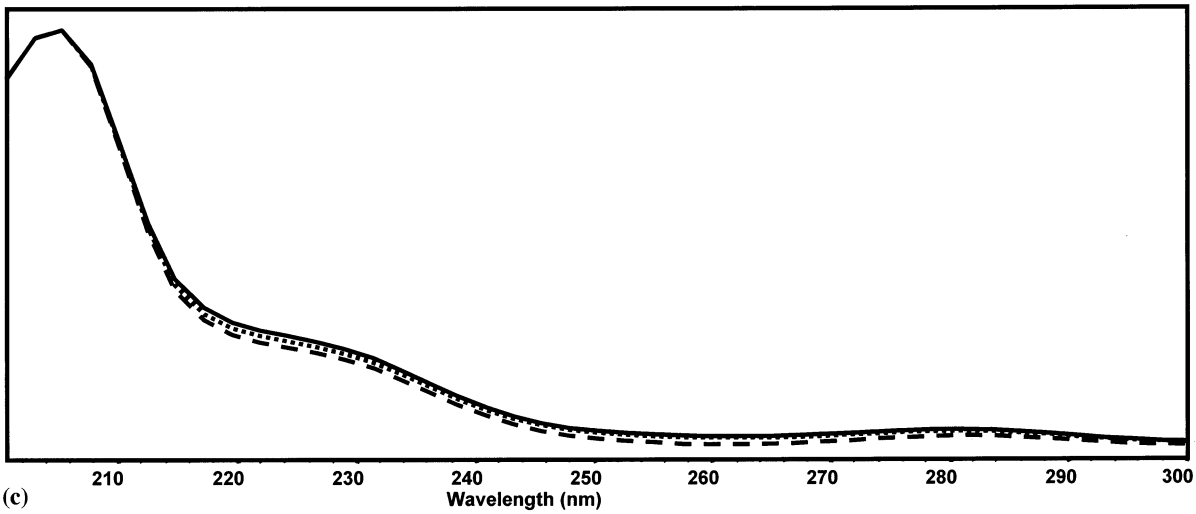
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Fig. 3.

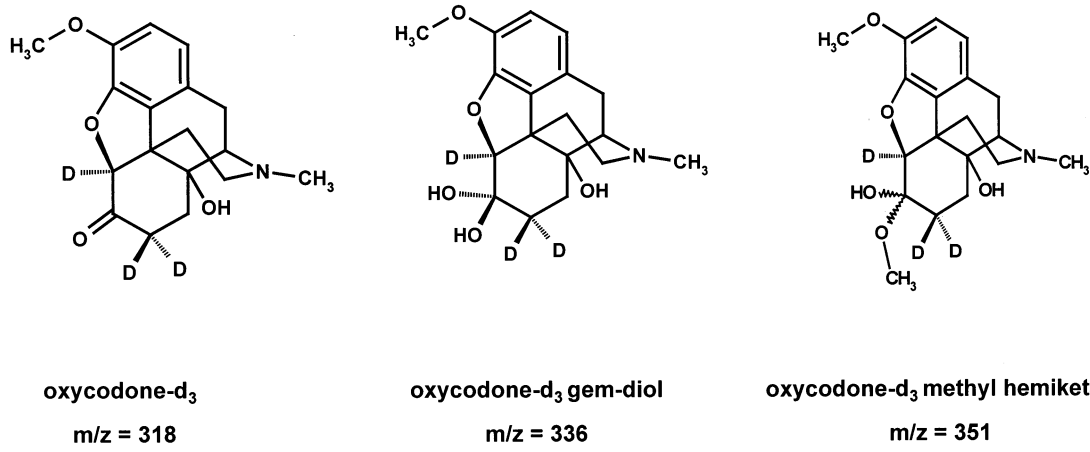

Fig. 4. Structures of the tri-deuterated species of oxycodone (m/z = 319, $M+1$) eluting in the peak front: oxycodone-d_3, tri-deuterated oxycodone gem–diol, (m/z = 337, $M+1$) and tri-deuterated oxycodone methyl hemiketal, (m/z = 351, $M+1$).

column temperature of 60°C, fronting was practically eliminated and previously undetected substances were separated from oxycodone for the first time. These were later identified to be the primary process impurities 14-hydroxycodeinone and 6α-oxycodol (7,8-dihydro-14-hydroxycodeine) [9]. Chromatography at 80°C further increased the symmetry of the oxycodone peak, but k' became unacceptably low (chromatogram not shown).

At 0°C, equilibria between three of the species was sufficiently slow to allow their resolution by HPLC. The major component is oxycodone; the other two species (eluting at 4.8 and 7.5 min) remained to be identified. Slow interconversion between the many species (see Fig. 1) leads to the type of fronting observed at 30°C. At 60°C, the rate of interconversion has increased sufficiently to be classified as a 'fast reaction' during separation. Under such conditions, the observed retention of oxycodone is the time weighted average of the retention of the three species shown in Fig. 4.

3.2. Photodiode array analysis at different column temperatures

Oxycodone exhibits a typical UV spectrum for the opiates. The absorption maxima at 280 nm band, although usually attributed to absorption by saturated ketones [10], is of a different origin in opioids since it is also present in morphine and codeine, molecules which do not contain this functional group.

Photodiode array data was obtained during chromatographic runs at 0, 30, and 60°C (see Fig. 3a, b, and c, respectively). Spectra from 200 to 300 nm were collected, and those at the start of fronting and the middle and apex of the main peak were normalized and compared. For spectra collected during the 60°C run, there was no shift in the absorption maxima at 206–280 nm, but differences in absorbance in the 215–240 nm region were observed. We attributed these differences to instrumental artifacts or the normalization of the spectrum of a trace component in the prominent peak. At 30°C, the spectrum obtained for the leading edge of the peak was markedly different from that obtained for the spectrum obtained for the apex and tailing edge of the peak. This difference was even more pronounced for spectra collected when the separation was performed at 0°C in that each of the four spectra recorded at 4.8, 7.5, 8.5, and 10.0 min was unique. However, no structural information could be gleaned from these spectra and it did not add to our understanding of the chromatographic behavior of oxycodone in reversed-phase HPLC systems.

3.3. Keto–enol tautomererization

The hypothesis that keto–enol tautomerization may, in part, cause the fronting was tested. The

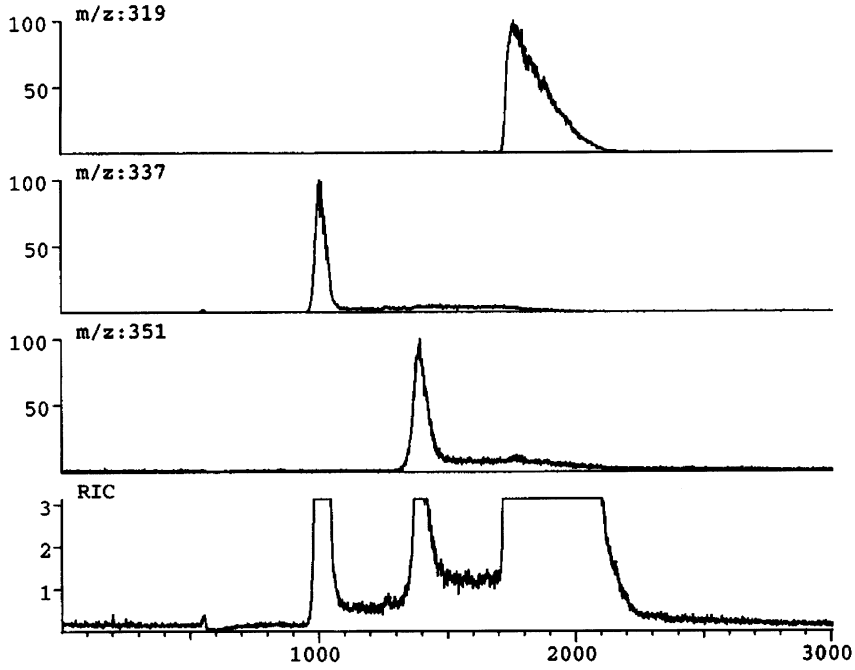

Fig. 5. LC/MS analysis and reconstructed ion chromatogram (RIC) of eluate at 0°C showing resolution of tri-deuterated oxycodone gem–diol, (m/z = 337, $M+1$), and tri-deuterated oxycodone methyl hemiketal, (m/z = 351, $M+1$) from the peak front of tri-deuterated oxycodone (m/z = 319, $M+1$). See also, 0°C–chromatogram in Fig. 2.

three protons α to the carbonyl were replaced with deuterium and the stability of tri-deuterated oxycodone was studied in acidified methanol (pH 1). In this experiment, 10 μl aliquots of the oxycodone deuteration reaction mixture were withdrawn, diluted to 1 ml with acidified methanol, and analyzed by mass spectrometry. No loss of deuterium was observed in samples stored at room temperature in acidified methanol for more than 1 week. Therefore, keto–enol tautomerization is not the cause of fronting of the oxycodone peak.

3.4. LC/MS analysis of eluate at different column temperatures

Deuterated oxycodone was dissolved in methanol and chromatographed on the μBondapak C_{18} column using a mobile phase without buffer and TEA so that we could obtain information regarding the identities of the two peaks we observed at the peak front of oxycodone. The use of this mobile phase eliminated the presence of non-volatile buffer as well as TEA, which greatly reduced the ionization efficiency of oxycodone and therefore the sensitivity of the mass spectrometer. MTBE was also eliminated from the mobile phase for these experiments. This modified mobile phase had no effect on fronting, but resulted in severe tailing of the oxycodone peak. This compromise was acceptable because it allowed us to obtain valuable mass spectral information.

The identities of the two unknown species in the peak front of tri-deuterated oxycodone (m/z = 31, $M+1$) were established as the tri-deuterated oxycodone gem-diol, (m/z = 337, $M+1$), and the tri-deuterated oxycodone methyl hemiketal, (m/z = 351, $M+1$) Fig. 4. The mass chromatograms and the reconstructed ion chromatograms (RIC) of these three species at column temperatures of 0, 30 and 60°C are shown, respectively, in Figs. 5–7. Comparison of the reconstructed ion chromatograms at the three

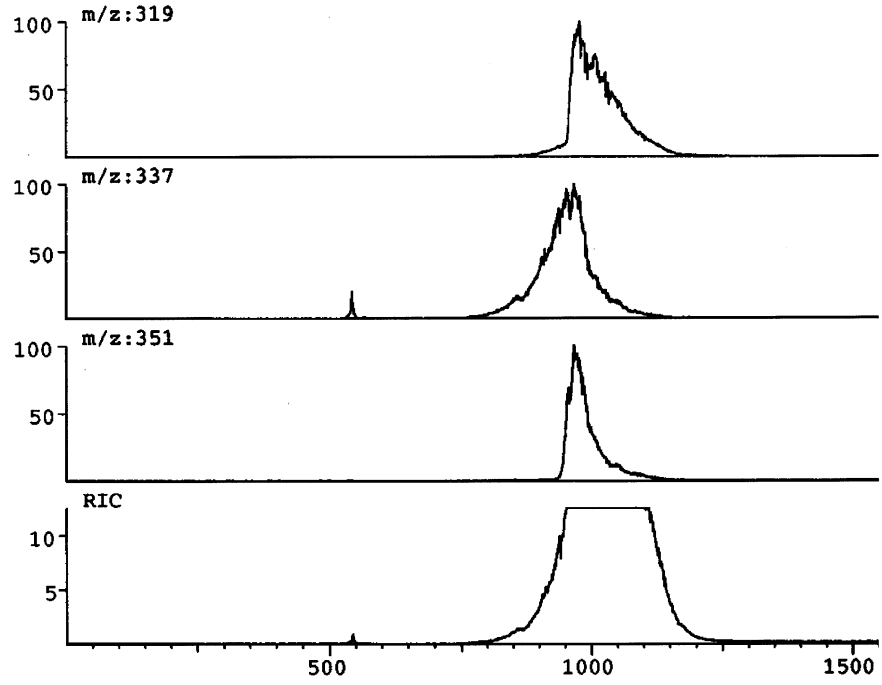

Fig. 6. LC/MS analysis and RIC of eluate at 30°C showing the relative contributions of tri-deuterated oxycodone gem–diol and hemiketal to the peak front of tri-deuterated oxycodone. See also, 30°C–chromatogram in Fig. 2.

temperatures demonstrates that as the temperature decreased, the rate of interconversion among oxycodone and its solvent adducts was sufficiently slowed, relative to the time scale of the separation, to allow their resolution from oxycodone and each other.

4. Discussion

Oxycodone (I) is a complex molecule, which, in addition to a reactive ketone moiety, contains phenyl methyl ether, a tertiary amine, a strained dihydrofuran ring, and a tertiary alcohol. In mobile phases containing water and methanol, the ketone can establish complex, acid-catalyzed keto–enol (IIa, b) [11], ketone–gemdiol (III) [12,13], and ketone–hemiketal (IV) [14,15] equilibria as illustrated in Fig. 1.

Nagase et al. [16] observed facile enol formation during the synthetic modification of oxycodone. Our experiments with tri-deuterated oxycodone, however, demonstrated that oxycodone does not enolize in acidified methanol, and therefore structures IIa and IIb do not contribute to the fronting of the oxycodone peak observed during reversed-phase chromatography. Caldwell et al. observed the hydration of the C-6 ketone during an NMR experiment [17,18]. Formation of the gem–diol and hemiketal is the result of the addition of H_2O and methanol, respectively, to the C-6 ketone of tri-deuterated oxycodone. The gem–diol and the methyl hemiketal tend to exist only in aqueous solutions in equilibrium with oxycodone, and are not degradation products. Either one or both of these equilibrium processes could contribute to the extensive fronting observed during the reversed-phase HPLC analysis of oxycodone. The interconversion of these species is temperature dependent. Decreasing the temperature slowed the rate at which the species equilibrated, enabling the separation of these two solvent adducts. As the temperature was increased, the rate of interconversion increased as shown by the gradual merging of the three peaks (Fig. 2).

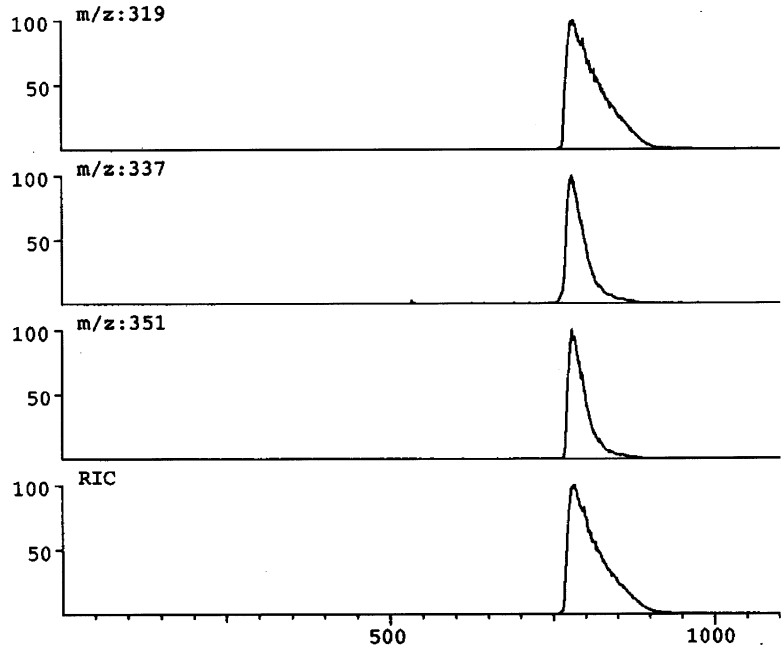

Fig. 7. LC/MS analysis and RIC of eluate at 60°C demonstrating similar retention times for oxycodone, the gem–diol, and hemiketal at increased temperature. See also, 60°C–chromatogram in Fig. 2.

The mass chromatograms also show that equilibration of these three species continues during the chromatographic process, even at 0°C. Inspection of the expanded-scale mass chromatogram of oxycodone (m/z = 319) showed that reversion of the adducts through loss of solvent also takes place as evidenced by elution of the m/z = 319 peak before the main oxycodone peak. A close inspection of the mass chromatograms shows that although the equilibrium was slowed sufficiently to allow each species to elute as distinct peaks, there was still a detectable amount of interconversion occurring (Fig. 7). At 60°C the kinetics of the interconversions are so rapid with respect to their residence time on the column that they appear as one peak.

The differences in the UV spectra for the three peaks was the first indication that we were dealing with a complex system. The small differences and lack of structural information, however, made it difficult to draw any conclusions. It has now been established that these differences stem from the differences in the chemistries of the C-6 carbon of the three molecules.

5. Conclusions

This investigation clearly demonstrates that poor peak shapes can result from unexpected analyte–mobile phase/solvent reactions. Specifically, these results show that the equilibrium addition of two widely used HPLC solvents —water and methanol— to the C-6 ketone of oxycodone results in the formation of a gem–diol and methyl hemiketal during chromatography and that the solvent adducts that formed as a result of these equilibria are responsible for peak fronting. Elevating the column temperature was sufficient to minimize fronting by increasing the rate of the equilibration kinetics. Such chromatographic anomalies are reminders that chromatographers need to consider not just interactions between the analyte and the stationary phase and the possibility of on-column reactions, but also potential interactions of the analyte with the mobile phase.

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