ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
14 (1996) 1663–1674

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Profiling impurities and degradants of butorphanol tartrate using liquid chromatography/mass spectrometry and liquid chromatography/tandem mass spectrometry substructural techniques[1]

Kevin J. Volk[a,*], Steven E. Klohr[a], Robyn A. Rourick[a], Edward H. Kerns[a], Mike S. Lee[b]

[a]*Bristol-Myers Squibb Pharmaceutical Research Institute, 5 Research Parkway, P.O. Box 5100, Wallingford, CT 06492, USA*

[b]*P.O. Box 4000, Princeton, NJ 08543, USA*

Received for review 23 January 1996; revised manuscript received 15 April 1996

Abstract

A rapid and systemic strategy based on liquid chromatography/mass spectrometry (LC/MS) profiling and liquid chromatography/tandem mass spectrometry (LC/MS/MS) substructural techniques was utilized to elucidate the degradation products of butorphanol, the active ingredient in stadol® NS. This strategy integrates, in a single instrumental approach, analytical HPLC, UV detection, full-scan electrospray mass spectrometry, and tandem mass spectrometry to rapidly and accurately elucidate structues of impurities and degradants. In these studies, several low-level degradation products were observed in long-term storage stability samples of bulk butorphanol. The resulting analytical profile includes information on five degradants including molecular structures, chromatographic behavior, molecular weight, UV data, and MS/MS substructural information. The degradation products formed during long-term storage of butorphanol tartrate included oxidative products proposed as 9-hydroxy- and 9-keto-butorphanol, norbutorphanol, a ring-contraction degradant, and Δ1, 10*a*-butorphanol. These methodologies are applicable at any stage of the drug product cycle from discovery through to development. This library of butorphanol degradants provides a foundation for future development work regarding product monitoring, as well as a useful diagnositc tool for new degradation products.

Keywords: Butorphanol; Degradants; LC/MS; Tandem mass spectrometry

1. Introduction

Butorphanol, the active ingredient in Stadol® NS, is formulated as an intranasal analgesic

* Corresponding author. Tel.: (+1) 203-284-6812; Fax: (+1) 203-284-6137.

[1] Presented at the Analysis and Pharmaceutical Quality Section of the Tenth Annual American Association of Pharmaceutical Scientists Meeting, November, 1995, Miami, Florida, USA.

PII S0731-7085(96)01841-9

product and is currently used in the treatment of pain associated with post-surgical situations, dental intervention, and migraine [1–3]. During the course of routine stability studies, several low-level degradants were observed in the HPLC/UV chromatogram. To rapidly obtain detailed structural information about the trace level impurities and degradants observed in the bulk drug substance, liquid chromatography/mass spectrometry (LC/MS) and liquid chromatography/tandem mass spectrometry (LC/MS/MS) strategies were developed based on previous methods for the rapid and systematic elucidation of drug metabolites in physiological fluids and natural products in crude and partially purified extracts [4–7]. Analyses combined optimized HPLC separation conditions on-line with an electrospray/MS interface to obtain molecular weight information from the full-scan mass spectra (LC/MS) and structural information from the tandem mass spectra (LC/MS/MS). Using these methodologies, structural and substructural data for trace butorphanol-related components in the formulated drug were obtained rapidly and systematically without prior fractionation. Furthermore, chromatographic resolution of co-eluting or unresolved components is not required to obtain product ion data for structural analysis, due to the mass-resolving capability of mass spectrometry. As a result, this strategy has permitted the development of an LC/MS-based butorphanol degradant database which includes: chromatographic characteristics using a standard HPLC system, molecular weight, and collision-induced dissociation (CID) MS/MS product ion spectra.

During the course of drug development, the bulk drug and drug formulation are studied under different stress conditions such as temperature, humidity, acidity, basicity, oxidizing conditions, and light. The stressing conditions which may cause drug degradation are utilized to validate the analytical monitoring methods and serve as predictive tools for future formulation and packaging studies [8]. Traditional methods involving process scale-up, isolation, and purification of trace components such as degradants and impurities are expensive and time-consuming. The use of methods such as those described here permit the rapid cataloging and identification of potential degradants and are attractive alternatives that serve to decrease the drug product development cycle time.

In addition to previously described studies [4,5] involving the identification of trace level taxanes related to paclitaxel in natural and process-related extracts using LC/MS and LC/MS/MS methodologies, these laboratories have also been involved in using this same LC/MS and LC/MS/MS strategy to identify degradation products in long-term storage stability samples (solutions) of butorphanol tartrate. In this paper, the profile informaton obtained from the LC/MS and LC/MS/MS analyses of a long-term storage stability sample will be discussed.

2. Experimental

2.1. Sample preparation

Long-term storage stability samples of butorphanol tartrate were obtained as the formulated product (10 mg ml^{-1} in water). The samples were aged for approximately 170 weeks at 30°C in the dark.

2.2. Chromatography

A Hewlett Packard 1090M HPLC system (Avondale, PA) was used with UV detection at 280 nm. A modified version of an assay for butorphanol tartrate was used and consisted of a narrow-bore phenyl column (2.0 mm × 250 mm, 5 μm; Keystone Scientific, Bellefonte, PA) with mobile phase A: 2 mM NH_4OAc, pH 4.8; and mobile phase B: acetonitrile. A gradient was performed from 65/35 (A/B) to 50/50 in 10 min, followed by a 10 min hold at 50/50 at a flow rate of 0.35 ml min^{-1} at ambient temperature. 3 μl of the aqueous formulated product was injected.

2.3. Electrospray mass spectrometry

A PE-SCIEX (Thornhill, Ont, Canada) API III tandem quadrupole mass spectrometer equipped with an ionspray (nebulizer-assisted electropsray)

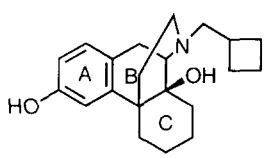


Fig. 1. Structure of butorphanol.

interface was used on-line with the HPLC system described above. Since the ionspray interface operates most effectively as flow rates less than 100 μl min^{-1}, the eluent from the UV detector was spilt approximately 1:3 prior to the interface. The split ratio was easily regulated by adjusting the length of the restriction line (fused-silica capillary with 50 μm i.d. and 160 μm o.d.). LC/MS experiments were performed while scanning from m/z 150 to 1000 at a scan rate of 2 s per scan. For LC/MS/MS substructural studies, the parent ions were selected in the first quadrupole mass analyzer and transmitted into the second quadrupole (collision cell) with a collision energy of 50 eV and an argon collision gas density of 400×10^{12} molecules cm^{-2}.

3. Results and discussion

During the course of conducting routine stability studies on butorphanol nasal spray (10 mg ml^{-1}), several very low-level degradants were observed. Most of the time these chromatographic peaks were sporadic, near the detection limit, and often disappeared upon retest. However, in one case several peaks were still observed after retest. Due to the high sensitivity of mass spectrometry, it has been found that implementation of LC/MS profiling methods is particularly advantageous for the rapid characterization of impurities and low-level degradants [4,5,8,9]. The LC/MS profile method used in these studies provided reasonable resolution and reproducible relative retention times (RRT; relative to butorphanol). This has resulted in the development of a database containing chromatographic and structural information about butorphanol-related impurities and degradants and the ability to transfer data and information to collaborating laboratories which may not possess LC/MS capabilities. In addition,

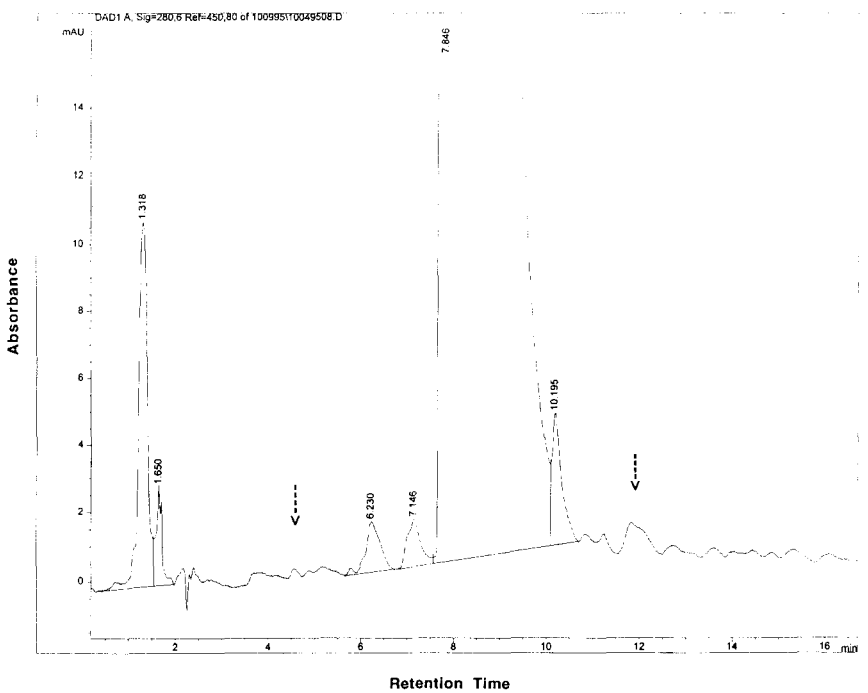


Fig. 2. HPLC/UV chromatogram (280 nm) of a 3 μl injection of an aqueous LTSS sample of butorphanol tartrate. The two arrows indicate the location of two very low-level degradants which were below the cutoff for LC/UV monitoring.

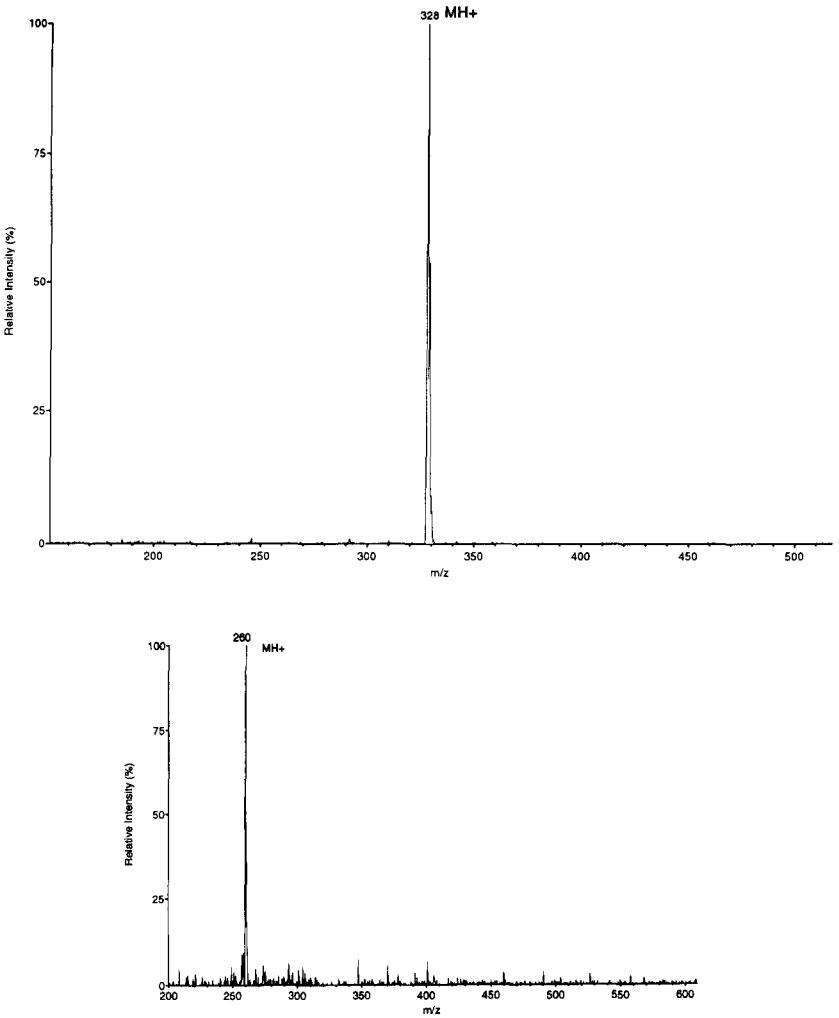


Fig. 3. Full-scan mass spectra of (A) butorphanol, indicitaing an MH^+ ion at m/z 328, RT 8.2 min, obtained during profiling studies of a LTSS sample of butorphanol tartrate; (B) the early butorphanol degradant eluting at 4.6 min, with an MH^+ ion at m/z 260, indicating the degradant to be norbutorphanol.

the reverse-phase HPLC conditions also provided a general measure of the lipophilicity of each compound, useful for interpretation of substructural differences between related degradants.

The structure of butorphanol is shown in Fig. 1. Fig. 2 illustrates the HPLC/UV chromatogram of a long-term storage stability (LTSS) sample of aqueous butorphanol tartrate. Several low-level components were observed in the HPLC/UV chromatogram at retention times of 6.2 min, 7.1 min, and 10.2 min. Two other very low-level degradants (indicated by the arrows), were below the LC/UV cutoff criteria. However, each was observed during the LC/MS profiling studies at their retention times of 4.6 min and 11.8 min. The elution order of the three early-eluting degradants indicated that they were less lipophilic than butorphanol.

The electrospray LC/MS interface utilized in these studies uses an extremely soft ionization process and produces primarily pseudo-molecular ions such as MH^+, providing definitive molecular weight information. Fig. 3A illustrates the full-scan mass spectrum of butorphanol obtained dur-

ing the LC/MS degradant profiling study of the LTSS sample. The ion at m/z 328 (MH^+) for butorphanol clearly indicates a molecular weight of 327 Da. The difference between the molecular weight of butorphanol and its long-term storage degradants is indicative of the substructural differences between the compounds. For example, comparison of the molecular weight difference between butorphanol (MW 327) and the degradant at 4.6 min (Fig. 3B), MW 259, demonstrates a molecular weight difference of 68 Da, indicative of the absence of the cyclobutane side-chain in the 4.6 min degradant. Based on the structure of butorphanol shown in Fig. 1, one potential degradative process could involve N-dealkylation resulting in loss of the side-chain to yield norbutorphanol. In fact, information obtained on-line during LC/MS profile studies of the LTSS sample indicated that the 4.6 min component (first arrow) has the same retention time and molecular weight as a standard of norbutorphanol.

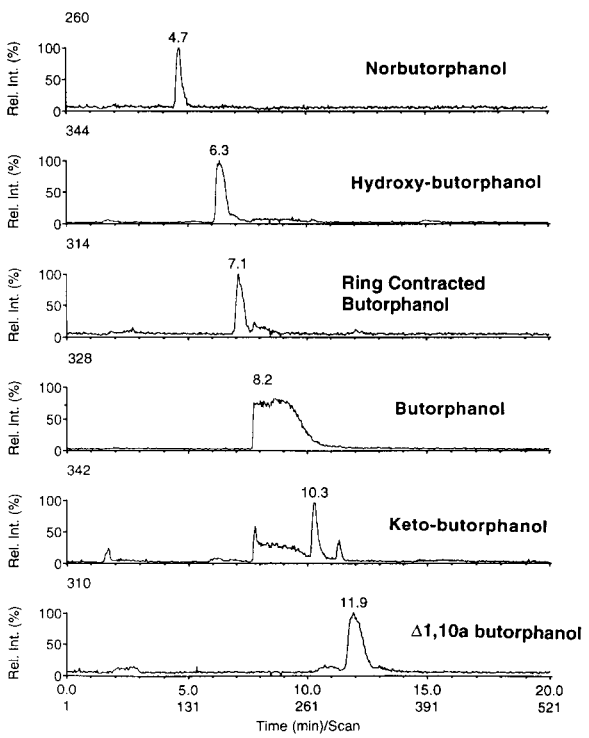


Fig. 4. LC/MS mass chromatograms (extracted ion current profiles) of butorphanol and its long-term storage degradants (170 weeks at 30°C) at the m/z ratio of their MH^+ ion. The m/z ratios of the mass chromatograms are shown on the left of each trace.

The initial molecular weight information obtained during the examination of the full-scan mass spectra of each degradant at its respective retention time can be seen in Fig. 4 which illustrates the LC/MS mass chromatograms corresponding to the protonated molecular ions of butorphanol and its degradants observed in the LTSS sample. As can be seen in Fig. 4, molecular weight information was obtained for five degradants of butorphanol. The degradants were found to have molecular weights corresponding to M + 16 Da, M + 14 Da, M − 18 Da, M − 14 Da, and M − 68 Da, where M is the molecular weight of butorphanol. In LTSS samples one might expect degradative processes such as N-dealkylation to yield norbutorphanol (M − 68 Da), oxidative processes to yield hydroxy-butorphanol (M + 16 Da) and keto-butorphanol (M + 14 Da), and elimination reactions to yield a ring contraction (M-14 Da) or loss of water (M − 18 Da). Therefore, the molecular weights determined during the LC/MS profile of the LTSS sample degradants of butorphanol are not unexpected and rapidly provide consistent structural information for initial structure proposal and confirmation.

3.1. LC/MS/MS Structural Studies

When dealing with mixtures of trace components, as illustrated in the HPLC/UV chromatogram in Fig. 2 and the complementary mass chromatograms shown in Fig. 4, the production of only molecular ions is an ideal situation for molecualr weight confirmation since virtually all the ion current is consolidated into a pseudo-molecular ion. Molecular weight information coupled with chromatographic retention characteristics and diode array UV spectra can be of tremendous benefit to delineate impurities and degradants. When electrospray ionization is coupled on-line with tandem mass spectrometry (LC/MS/MS), detailed structural information for each component can be obtained [4,5,9]. In such profiling studies, one relies heavily on the MS/MS fragmentation "templates" of known analogs to

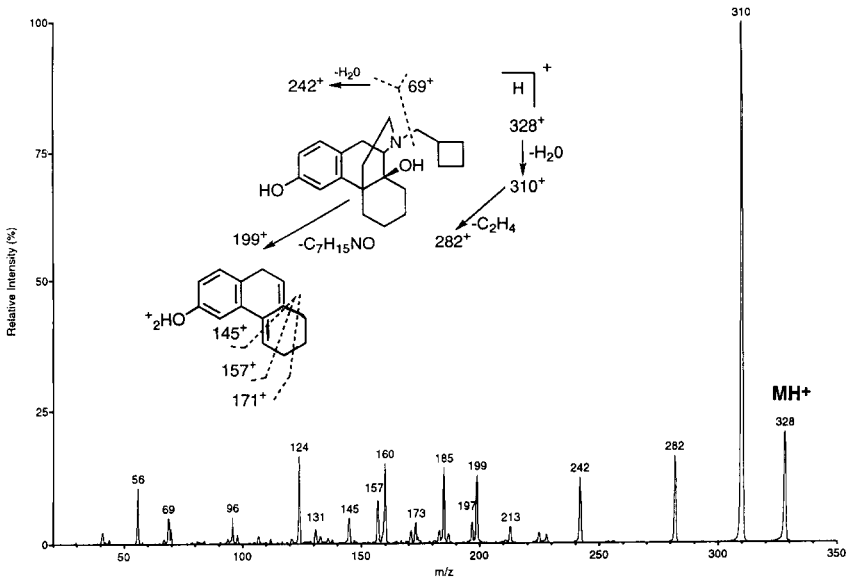


Fig. 5. MS/MS product ion spectrum of MH^+ ion of butorphanol at m/z 328. Product ions, neutral losses and their proposed correspondence to specific substructures are indicated.

assist in the structural determination of impurities and degradation products. Therefore, in addition to chromatographic retention characteristics, molecular weight information, and insights regarding chemical lability, the structures of the butorphanol LTSS sample degradants were proposed based on the comparison of their MS/MS fragmentation patterns with those of butorphanol and norbutorphanol which were used as substructural templates. Interpretation of the structures of unknown degradants proceeded by the association of specific product ions and neutral losses with specific substructures. This MS/MS comparative method is based on the premise that the targeted degradants are expected to retain much of the original butorphanol structure (Fig. 1). Therefore, burtorphanol-related degradants would be ecpected to undergo similar fragmentations to butorphanol and the norbutorphanol standard. Common MS/MS product ions and neutral losses observed in the product ion spectrum of butorphanol and the product ion spectra of the degradants were evidence for common substructures and differences were indicative of variance in those substructures.

Fig. 5 illustrates the MS/MS products ion spectrum of butorphanol at m/z 328 ($M + H^+$). A facile loss of water yields an ion at m/z 310, one of the primary product ions, followed by either sequential loss of 28 Da corresponding to loss of ethene (C_2H_4) to yield a product ion at m/z 282 or loss of the cyclobutane side-chain to yield the product ion at m/z 242. Other diagnostic product ions as shown in Fig. 5 are at m/z 199, indicative of the butorphanol (A–B–C ring) core, and subsequent fragmentations to produce ions at m/z 171, 157, and 145. The product ion spectrum is a unique fingerprint for each compound and can be used for structure elucidation purposes as well as confirmation of the presence of a suspected component. An example of how LC/MS/MS structural profiling can be used to confirm the presence of a particular compound can be seen in Fig. 6A, which illustrates the product ion spectrum of the m/z 260 $(M + H)^+$ ion of the 4.6 min degradant. Fig. 6B illustrates the product ion spectrum of a reference standard of norbutorphanol at m/z 260. Comparison with the product ion spectra of the butorphanol degradant in Fig. 6A indicates that the 4.6 min degradant is indeed norbutorphanol.

Based on its full-scan mass spectrum (MW 341) and its UV–Vis diode array spectrum, the 10.2 min component which elutes in the tail of butorphanol may correspond to keto-butor-

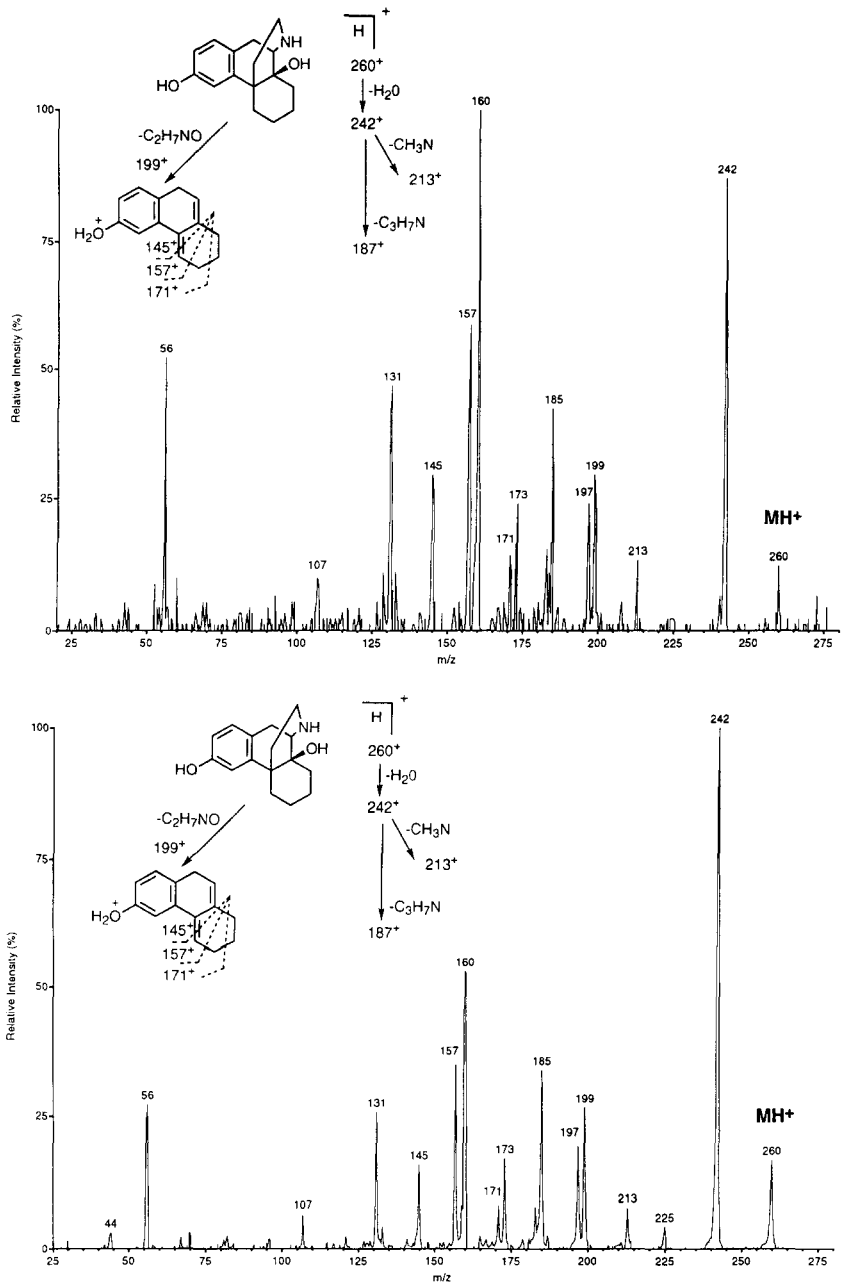


Fig. 6. (A) MS/MS product ion spectrum of MH^+ ion at m/z 260, corresponding to the 4.6 min butorphanol degradant indicated by the first arrow shown in Fig. 2. (B) MS/MS product ion spectrum of the MH^+ ion of a norbutorphanol reference standard at m/z 260.

phanol. A comparison of the diode array UV–Vis spectra of the 10.2 min degradant and butorphanol is shown in Figs. 7A and 7B respectively. The UV–Vis spectra of the degradant indicate a "red shift", suggesting extended conjugation of this component with respect to butorphanol. The LC/MS/MS product ion spectrum of this component is shown in Fig. 8. The product ion spectrum

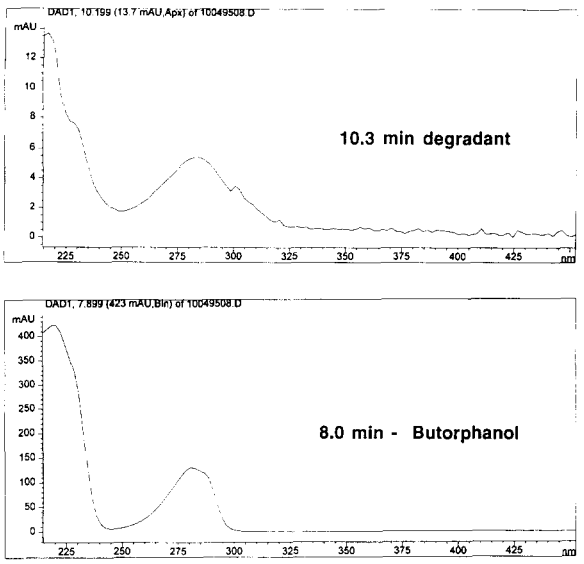


Fig. 7. Diode array UV–Vis spectrum of (A) the 10.3 min degradant of butorphanol; (B) butorphanol at a retention time of 8.0 min. Note the "red shift" indicating extended conjugation in the 10.3 min degradant.

contains a neutral loss of 68 Da from the product ion at m/z 324, which indicates that the side-chain is intact. However, a key product ion at m/z 199 indicative of the butorphanol (A–B–C ring) core is noticeably absent in the product ion spectrum of the degradant. Instead, a product ion at m/z 211 is observed. Consecutive fragmentation can produce the proposed cleavages (C-ring) to produce ions at m/z 185 and 157, suggesting oxidation at the 9-position to a carbonyl. Based on discussions with medicinal chemists within Bristol-Myers Squibb, the 9-position is the most likely location for oxidation to occur. As a result, this degradant was proposed to be 9-keto-butorphanol. As can be seen in Fig. 4, baseline signal liftoff for the ion trace at m/z 342 occurs during ionization of butorphanol. The response during this retention time window is not due to another component but is due to overloading of the ion source and detector, and subsequent clustering effects form overloading during the ionization of the major component, butorphanol. A minor peak, which is not labeled in the mass chromatograms, elutes after ketobutorphanol at about 11.2 min. The presence of this component in the mass chromatogram and its full-scan mass spectrum were not explored since it appears to be present well below the 0.1% level in the LC/UV chromatogram.

The LC/MS/MS product ion spectrum of the 6.3 min component, assigned by molecular weight

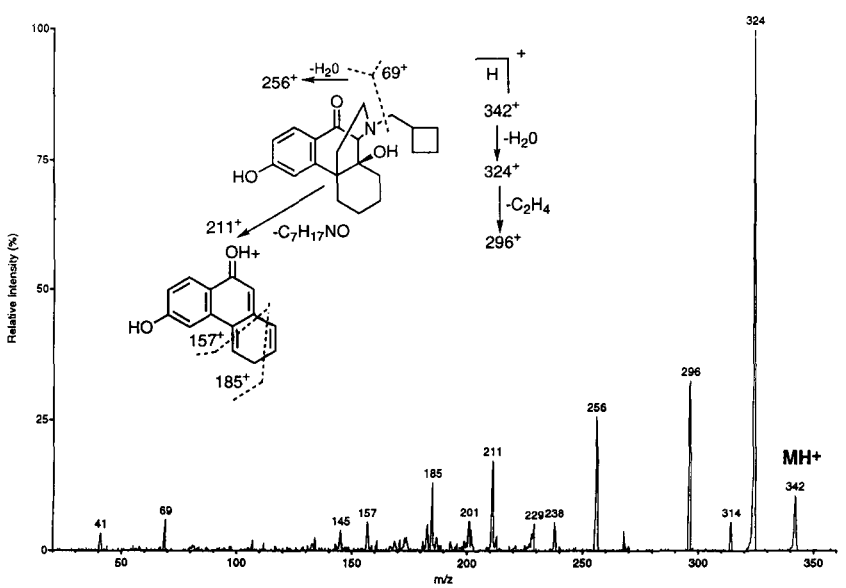


Fig. 8. MS/MS product ion spectrum of the MH^+ ion of the keto-butorphanol degradant at m/z 342 observed in the LTSS sample at 10.2 min.

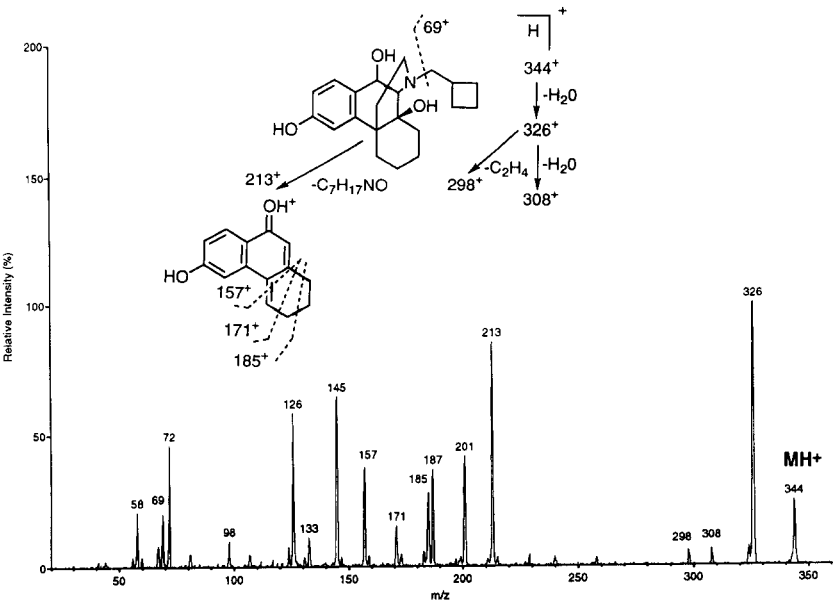


Fig. 9. MS/MS product ion spectrum of the MH^+ ion of the hydroxy-butorphanol degradant at m/z 344 observed in the LTSS sample at 6.3 min.

information as hydroxy-butorphanol, is shown in Fig. 9. N-oxide formation is another potential oxidative degradation mechanism which could account for a degradant which is 16 Da greater than the parent drug. However, in these studies a standard of the N-oxide was available but did not co-chromatograph or have the same LC/MS/MS product ion spectrum as that of the degradant. As can be seen in Fig. 9, two neutral losses of water are observed from the precursor ion at m/z 344, suggesting hydroxylation of an aliphatic substructure of the molecule. The product ion spectrum also contains a neutral loss which indicates that the side-chain is intact. As was discussed for keto-butorphanol, a key product ion at m/z 199 indicative of the butorphanol (A–B–C ring) core is also absent in the product ion spectrum of hydroxy-degradant. Instead, a product ion at m/z 213 is observed. Consecutive fragmentation can produce the propesed cleavages (C-ring) to produce ions at m/z 185 and 157, supporting oxidation at the 9-position. The presence of 9-hydroxy-butorphanol is not unusual since further oxidation would result in the formation of 9-keto-butorphanol. As a result of these studies, the 6.3 min degradant was proposed as being 9-hydroxy-butorphanol.

The M-14 Da component which elutes prior to butorphanol at 7.1 min appears to be a degradant formed as a result of a ring contraction. It is not clear from the LC/MS/MS product ion spectrum if the ring contraction has occurred in the B- or C-rings. Product ions corresponding to loss of water at m/z 296 followed by loss of the side-chain at m/z 228 are observed. Subsequent loss of C_2H_5N from m/z 228 produces a product ion at m/z 185, indicating contraction of either the B- or C-rings. The ion at m/z 185 is complementary to the product ion at m/z 199 observed in the product ion spectrum of butorphanol corresponding to the core (Fig. 5). As a result, the LC/MS and LC/MS/MS studies localized the modification (ring contraction) to either the B- or C-rings. Additional spectroscopic analyses are planned to further explore the structure of this degradant.

The late-eluting degradant at 11.9 min (indicated by the arrow in Fig. 2) has a molecular weight of 309 Da, as determined by the LC/MS profile. The chromatographic retention and molecular weight are consistent with an elimination product corresponding to the loss of water. The produce ion spectrum of this degradant is

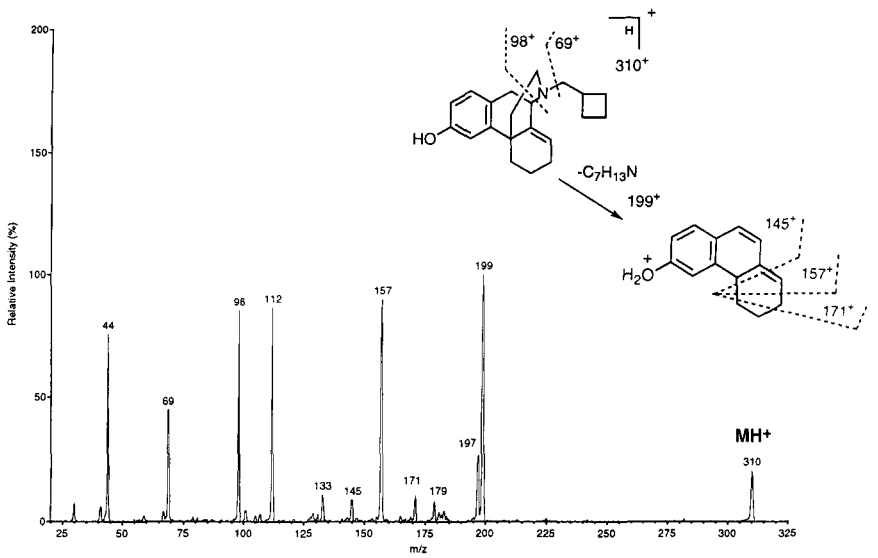


Fig. 10. MS/MS product ion spectrum of the MH^+ ion of the Δ1, 10*a*-butorphanol degradant at *m/z* 310 observed in the LTSS sample at 11.9 min.

shown in Fig. 10. Noticeably absent in the product ion spectrum of the 11.9 min degradant is the neutral loss of water from the precursor ion at *m/z* 310. The product ion spectra of butorphanol, as well as of all the other observed degradants which contain the hydroxyl group on the aliphatic C-ring, demonstrate a facile loss of water. The product ion at *m/z* 199 corresponding to butorphanol core minus water is observed as well as characteristic cleavages of the C-ring. This information, coupled with the late elution time clearly indicates that this degradant does not contain the 10*a* hydroxy group and it has been proposed as being Δ1, 10*a*-butorphanol.

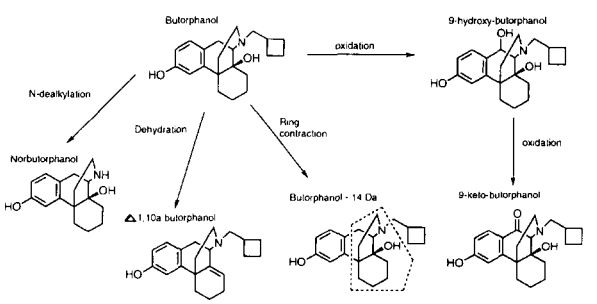


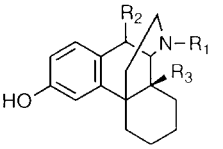
Fig. 11. Degradation pathway of butorphanol tartrate in LTSS samples determined by LC/MS profiling. Dashed area indicates substructure involved in proposed ring contraction.

Based on the information obtained during these LC/MS and LC/MS/MS studies, the 170-week-old aqueous LTSS samples exhibited excellent stability. The degradation pathway and LC/MS profile data are summarized in Fig. 11 and Table 1, and indicate the degradative processes observed for the LTSS samples of butorphanol tartrate. In less than 1 day, the structure elucidation strategy described in this paper provided detailed information regarding trace level components in the stability samples. When this information was correlated with known or predicted chemically labile portions of the molecule, structures were rapidly proposed. As a result, synthesis of the proposed degradants and subsequent confirmation occurred much sooner than with the traditional structure elucidation strategies.

4. Conclusions

The high sensitivity of mass spectrometry is particularly advantageous for application to samples which contain trace impurities and degradants, a situation frequently encountered in pharmaceutical discovery and development research. A strategy involving the use of LC/MS

Table 1
Butorphanol degradants from long-term stability storage of an aqueous formulation identified using LC/MS profiling



Proposed structure	RRT	Molecular weight	R1	R2	R3
Norbutorphanol	0.58	259	H	H	OH
Hydroxy-butorphanol	0.78	343	$-CH_2(CH_7)$	OH	OH
Ring-contracted butorphanol	0.90	313	$-CH_2(C_4H_7)$	H	OH
Butorphanol	1.0	327	$-CH_2(C_4H_7)$	H	OH
Keto-butorphanol	1.3	341	$-CH_2(C_4H_7)$	=O	OH
Δ1, 10*a*-butorphanol	1.5	309	$-CH_2(C_4H_7)$	H	H

profiling and LC/MS/MS substructural analysis has been shown to be capable of providing a highly sensitive and specific method for rapidly obtaining molecular weight and structural information concerning low-level impurities and degradants.

Degradation products were elucidated on the basis of their chromatographic retention times using standardized HPLC conditions, molecular weight information obtained from the full-scan mass spectrum acquired during LC/MS profiling, and the product ion spectrum acquired during LC/MS/MS substructure analysis studies. The degradation products formed during long-term storage of butorphanol tartrate included oxidative products which were proposed as 9-hydroxy and 9-keto-butorphanaol, an N-dealkylated component which was confirmed as norbutorphanol, a ring contraction component (location either B- or C-rings), and an elimination product proposed as Δ1, 10-butorphanol.

LC/MS and LC/MS/MS techniques can be used to facilitate the rapid analysis of samples based on the integration of bench-scale mixture analysis methodology (scale-up, fractionation and individual spectroscopic analysis) into a single on-line instrumental technique. This hyphenated instrumental technique provides multiple dimensions of information for structure elucidation which includes relative retention time, UV area percent for quantitative studes, UV–Vis spectra, molecular weight, and substructural information via tandem mass spectrometry. It has been found that this methodology permits the rapid and systematic characterization of trace level components, providing new opportunities to accelerate the drug development process. In addition, the proactive application of LC/MS profiling and LC/MS/MS substructural analysis in predictive forced degradation studies can also provide a foundation for future work involving the analysis of new degradation products, thereby positively impacting the drug development cycle.

Acknowledgments

The authors gratefully acknowledge the contributions of collaborators in this project. Ira E. Rosenberg and Jerry R. Allison encouraged and supported these studies and provided valuable resources. Discussions with Henry Wong were helpful in proposing certain potential degradant structures. Terry Wilson graciously supplied the long-term storage stability samples of butorphanol tartrate.

References

[1] R.C. Heet, Drugs, 16 (1978) 473–475.

[2] M. Lippman, Curr. Ther. Res. Clin Exp., 22 (1977) 276–281.
[3] J.F. Zeedick, Curr. Med. Res. Opinion, 6 (1979) 178–182.
[4] E.H. Kerns, K.J. Volk, S.E. Hill and M.S. Lee, J. Nat. Prod., 57 (1994) 1391–1403.
[5] E.H. Kerns, K.J. Volk, S.E. Hill and M.S. Lee, Rapid Commun. Mass Spectrom., 9 (1995) 1539–1545.
[6] M.S. Lee, D.J. Hook, E.H. Kerns, K.J. Volk and I.E. Rosenberg, Biol. Mass Spectrom, 22 (1993) 84–88.
[7] K. Yeleswaram, K.J. Volk, R.A. Rourick, J.D. Catt, R.J. Mattson, K.B. McGoff and J.O. Knipe, Res. Commun. Mol. Pathol. Pharmacol., 89 (1995) 27–44.
[8] X. Qin, D.P. Ip, K.H. Chang, P.M. Dradransky, M.A. Brooks and T. Sakuma, J. Pharm. Biomed. Anal., 12 (1994) 221–233.
[9] C.E. Parker, J.R. Perkins, K.B. Tomer, Y. Shida, K. O'Hara and M. Kono, J. Am. Soc. Mass Spectrom., 3 (1992) 563–574.