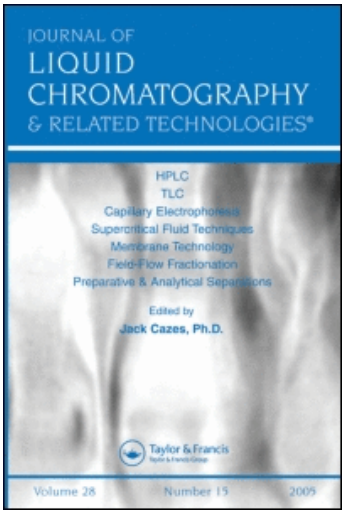


## Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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**To cite this Article** Wood, George C. , Iyer, Meera R. , Geller, Arthur M. , Fleischner, Albert M. and Sheth, Bhogi B.(1998) 'A High Pressure Liquid Chromatography Assay Method for Analysis of Deslorelin and Benzyl Alcohol in Deslorelin Injection', Journal of Liquid Chromatography & Related Technologies, 21: 14, 2183 — 2190
**To link to this Article: DOI:** 10.1080/10826079808006617
**URL:** http://dx.doi.org/10.1080/10826079808006617

J. LIQ. CHROM. & REL. TECHNOL., 21(14), 2183–2190 (1998)

# A HIGH PRESSURE LIQUID CHROMATOGRAPHY ASSAY METHOD FOR ANALYSIS OF DESLORELIN AND BENZYL ALCOHOL IN DESLORELIN INJECTION

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## ABSTRACT

Deslorelin is a nonapeptide drug used for precocious puberty in young women. A reversed phase HPLC procedure was developed for the analysis of deslorelin and benzyl alcohol in a parenteral solution dosage form. Both the nonapeptide drug (deslorelin) and the preservative (benzyl alcohol) were determined from a single injection. The separation and quantitation were achieved on a $C_{18}$ column at ambient temperature using a mobile phase of 70:30 v/v aqueous

phase:acetonitrile containing sodium hexylsulfonate and triethylamine at pH 3. The wavelengths for spectrophotometric detection were 275 nm for deslorelin and 254 nm for benzyl alcohol. The method showed linearity, precision and accuracy for deslorelin (2.93-550 mg/mL) and benzyl alcohol (0.059-11 mL/mL) over a large concentation range (0.6%-110% of the label concentration). The lower limits of quantitation for deslorelin and benzyl alcohol were 2.93 μg/mL and 0.059 μl/mL respectively.

## INTRODUCTION

Deslorelin (DES) is a nonapeptide and potent, synthetic agonist of Luteinizing Hormone Releasing Hormone (LHRH). DES inhibits the formation of female sex steroids and suppresses undesirable epiphyseal closure. Deslorelin[1-3] is indicated for the treatment of central precocious puberty in children, a condition of early sexual development accompanied by rapid bone aging that results in stunted growth. The DES formulation consisted of deslorelin (500 mg/mL), mannitol (100mg/mL) and benzyl alcohol (10 mg/mL) in sterile water for injection as a parenteral dosage form. Vials containing more than one dose of an injectable drug are required to contain a preservative. Benzyl alcohol was used as the preservative[4] and mannitol was incorporated as a peptide stabilizer.



Temperature accelerated stabiilty studies of new drug dosage forms require that both the active ingredient and preservative be quantitated. The method used here simultaneously quantitated deslorelin and benzyl alcohol. HPLC separation was achieved within 10 minutes at ambient temperature with sensitivity for both analytes in the μg/mL range. This method was found to be simple, selective, and reproducible when used for the accelerated stability studies or long term stability studies up to five years.

## MATERIALS AND METHODS

### Chemicals

Deslorelin was manufactured by Bachem Inc. (Torrance, CA).; benzyl alcohol, o-phosphoric acid and triethylamine were obtained from Fischer Scientific (Fairlawn, NJ); bovine serum albumin (BSA) was obtained from Sigma Chemical Corp (St. Louis, MO). 1-Hexane sulfonic acid was supplied

by Supelco, Inc.(Bellefont, PA) and mannitol were obtained from Mallinckrodt, Inc. (Paris, KY). HPLC grade acetonitrile and water (J. T. Baker, Phillipsburg, NJ) were used for the preparation of mobile phase.

## HPLC Equipment

The analysis was performed on a Waters HPLC system consisting of a model 510 pump, a model 712 WISP sample injector, a model 490E programmable multiwavelength UV-Vis detector, and a Baseline 810 chromatography workstation (all from Millipore Corporation, Milford, Massachusetts, USA).



## Chromatographic Conditions

Isocratic, reversed phase chromatography was performed at ambient temperatures (~22°C) using a $C_{18}$ column (250 x 4.6 mm i.d., Beckman). The mobile phase consisted of 70% HPLC grade water containing 0.1% sodium hexylsulfonate and 25 mM triethylamine (adjusted to pH 3.0 with 50% phosphoric acid) and 30% acetonitrile.

All separations were carried out at room temperature (approximately 20-22 degrees C) at a flow rate of 1.6 mL/min. The absorbances of deslorelin and benzyl alcohol were measured at 275 nm and 254 nm respectively.

## Standard Solutions Preparation

A set of ten standard solutions containing deslorelin and benzyl alcohol were prepared using the deslorelin stock solution and the benzyl alcohol stock solution. The deslorelin stock solution (approx. 750 µg/mL of deslorelin) was made by weighing approximatley 18.75 mg of deslerelin (purity 86.9%) to the nearest 0.01 mg and diluting the deslorelin with deslorelin diluent to 25 mL. The deslorelin diluent contained 100 mg/mL of mannitol and 1 mg/mL of bovine serum albumin in HPLC grade water. A benzyl alcohol stock solution was made by measuring 0.75 mL of benzyl alcohol into a 50 mL volumetric flask and diluting the benzyl alcohol to 50 mL with HPLC grade water to produce a stock solution containing 1.5% v/v of benzyl alcohol.

The deslorelin and benzyl alcohol stock solutions were mixed and diluted to produce standard solutions containing the various conentrtions of deslorelin and benzyl alcohol ranging from 110% to less than 1% of the label

concentrations. Deslorelin standards concentrations were to 2.9, 5.9, 12, 23.4,47, 94, 188, 200, 375 and 550 μg/mL. Benzyl alcohol standards concentrations were 0.059, 0.12, 0.23, 0.47, 0.94 , 1.88, 3.75, 4, 7.5, and 11 mL/mL.

### Sample Preparation and Injection

Peptides have a tendency to both aggregate and adsorb to surfaces. Small amounts of bovine serum albumin were added to prevent much of the aggregation and adsorption. One mg of bovine serum albumin was added to 1 mL of deslorelin sample solutions and the samples were vortexed for 20 seconds.

The HPLC injection volume was 25 μL. Triplicate injections were made of each standard solution and sample.



## RESULTS AND DISCUSSION

### Chromatography

The objective of this study was to develop a reliable, isocratic HPLC assay method for the analysis for deslorelin and benzyl alcohol in a parenteral product. The assay was used to test the stabiliity of the peptide and preservative in a deslorelin product undergoing accelerated stabilty testing at temperatures of -10 to +50°C. The drug peak was baseline separated from the preservative peak with a deslorelin retention time of 5.2 minutes and a benzyl alcohol retention time of 4 minutes (see Figure One).

Repeated analyses of freshly prepared standard solutions gave reproducible results on three separate days. Day to day variation of the assay ranged from 11%CV at the lower limit of detection to 2 %CV at the upper limit of detection.

Normally, drug assays are developed for the range of 50-150 percent of the analyte concentration but this assay ranged from less than 1% to 110% of the labeled drug concentration. Extension of the deslorelin and benzyl alcohol range to the low concentrations was necessary to assure that small concentations of peptide degradation products, could be detected and separated by the assay, if they were present.

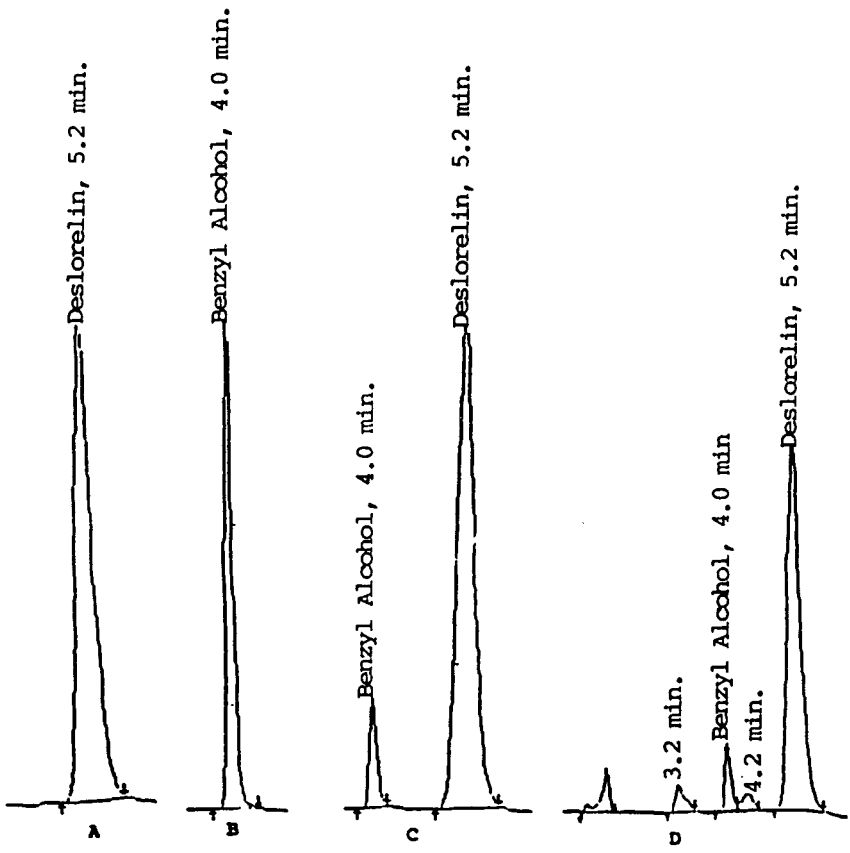


**Figure 1**. Chromatographs showing A: Deslorelin, B: Benzyl Alcohol, C: typical standard containing Deslorelin and Benzyl Alcohol, D: sample containing degradation products.

To produce a run time of less than 15 minutes for each injection, the mobile phase was adjusted to 70:30 aqueous solution:acetonitrile. Slight decreases in the 30% acetonitrile concentration of the mobile phase were found to have a significant increase in the retention time.

HPLC chromatograms of (a.) deslorelin, (b.) benzyl alcohol, (c.) a mixture of deslorelin and benzyl alcohol and (d.) a degraded accelerated stability sample are shown in Figure 1.

**Table 1**

**Within- and Between-Day Variability at Three Example Desorelin Concentrations**

| Within-Day Variability | | | Between-Day Variability | | |
|---|---|---|---|---|---|
| Conc. Spiked μg/mL | Conc. Found μg/mL (sd) | %CV | Conc. Spiked μg/mL | Conc. Found μg/mL (sd) | %CV |
| 2.93 | 4.07 (0.17) | 4.05 | 2.93 | 4.66 (0.51) | 11.03 |
| 23.44 | 21.83 (0.89) | 4.09 | 23.44 | 23.92 (1.72) | 7.18 |
| 93.75 | 90.91 (0.20) | 0.21 | 93.75 | 93.89 (2.44) | 2.6 |

Conc.=concentration, %CV=percent coefficient of variation, sd=standard deviation.,μg=microgram.



**Table 2**

| Within Day Variability | | | Between Day Variability | | |
|---|---|---|---|---|---|
| Conc. Spiked μL/mL | Conc. Found μL/mL (sd) | %CV | Conc. Spiked μL/mL | Conc. Found μL/mL (sd) | %CV |
| 0.058 | 0.06 (0.006) | 9.12 | 0.058 | .07 (0.007) | 11.08 |
| 0.93 | 1.02 (0.12) | 1.14 | 0.93 | 1.01 (0.010) | 0.96 |
| 4.00 | 4.21 (0.03) | 0.63 | 4.00 | 4.20 (0.03) | 0.70 |

Conc.=concentration, %CV=percent coefficient of variation, sd=standard deviation.,μL=microliter.

## Validation of HPLC Assay

### Linearity and sensitivity

Peak areas for each analyte standard solution were determined on three separate days. The standard calibration curves were linear over the range of 2.93 μg/mL-550 μg/mL for deslorelin and 0.059 μL/mL-11 μL/mL for benzyl alcohol. The coefficients of determination were 0.999-1.00 (deslorelin) and 0.995-0.997 (benzyl alcohol) when intercepts were forced through zero concentration of the analyte. The lower limits of quantitation were determined

to be 2.93 μg/mL for deslorelin and 0.059 μL/mL for benzyl alcohol. The maximum %coefficient of variation between days at the lower limits of quantitaion was 11% for both agents.

**Precision of the method**

Tables 1 and 2 summarize the within day and between variation of the method for deslorelin and benzyl alcohol. The precision of within day variation of deslorelin was 4% CVat 2.93 μg/mL and between day variation was 11%. The within day percent coefficient of variation for benzyl alcohol at 0.058 uL/mL was 9.12 % CV and 11.08 % CV for between days variation.

**Selectivity**

No interferring peaks were observed at the retention times for deslorelin and benzayl alcohol when a solution of (a) deslorelin (b) benzyl alcohol and (c) a mixture of deslorelin and benzyl alcohol were chromatographed using the described method. Benzyl alcohol degradation products did not interfere. Benzaldehyde had a retention time of 9.4 min and benzoic acid retention time was 6.0 min.



Deslorelin degradation peaks were noted at 3.2 min and 4.2 min in samples stored for three months at 50 degrees. These degradation peaks did not interfere with the quantitation for the analytes of interest. HPLC chromatograms of deslorelin, benzyl alcohol, a standard solution containing deslorelin and benzyl alcohol, and an accelerated stability sample showing degradation products are shown in Figure 1.

## CONCLUSIONS

An isocratic HPLC assay procedure was developed to determine deslorelin and benzyl alcohol concentrations in a deslorelin parenteral solution dosage form. The assays were linear, specific, and sensitive over a very large range for deslorelin and benzyl alcohol. A large concentration range for the standard solutions was used to detect potential degradation products in samples stored at temperatures for accelerated stability analysis. The lower limits of detection for both deslorelin and benzyl alcohol were 0.6% of labeled amount in the product. We concluded that the assay was a simple, sensitive, and a rapid HPLC procedure for the determination of both deslorelin and benzyl alcohol in parenteral solutions.

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Received December 1, 1996
Accepted December 18, 1996
Manuscript 4339