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Short communication

High-performance, purity-indicating liquid chromatographic method for the oxytocin antagonist, L-368,899.

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Abstract

This work describes the high-performance liquid chromatographic (HPLC) methodology used for the analysis of an oxytocin antagonist (L-368,899). The chromatography separates the drug from its major impurity (exo-isomer), four synthetic intermediates and two key raw materials. The method development process, which was required to achieve baseline separation of the eight components, reveals the subtleties and complexities involved in chromatographic method development. The optimized LC method was validated and found to be rugged and reproducible for the impurity profiling of this oxytocin antagonist drug.

Keywords: Pharmaceutical analysis; Oxytocin antagonists; ({Dimethyl-[amino(methylsulfonyl)butyramido]-bicycloheptanyl}methanesulfonyl)(methylphenyl)piperazine; Hormones; Tolylpiperazine derivatives

1. Introduction

L-368,899, [1S-((7,7-dimethyl-2-endo-(2S-amino-4 - (methylsulfonyl)butyramido) - bicyclo(2.2.1)heptan - 1 - yl)methanesulfonyl) - 4 - (2 - methyl - phenyl)-piperazine], is a synthetic tolylpiperazine derivative containing a primary amine function which has been evaluated as a non peptide competitive antagonist of oxytocin [1]. Oxytocin is the principal uterus-contracting and lactation stimulating hormone of the posterior pituitary gland [2], and is responsible for the initiation of labor, and resulting birth. An in vivo oxytocin antagonist has been shown to inhibit uterus contractions in monkeys and rats [3]. Therefore, an oxytocin antagonist has great potential to be of therapeutic benefit in the prevention and treatment of preterm labor.

In the development of new drugs, the analytical challenge is to quickly develop rugged methods for quality maintenance and impurity control. The determination of the purity and impurity profile of a drug candidate is important for establishing the continuing acceptability of subsequent lots in both toxicology and chemical studies. Ideally, the total amount of impurities, as well as the quantity of each individual impurity should be monitored. The quality assessment of a drug is typically made by a number of analytical tests [4]. One of the most common tests performed to determine drug quality by the pharmaceutical industry is the high-performance liquid chromatography (HPLC) impurity profile. The resulting chromatogram should have a homogeneous main peak and well-resolved peaks for the known impurities.

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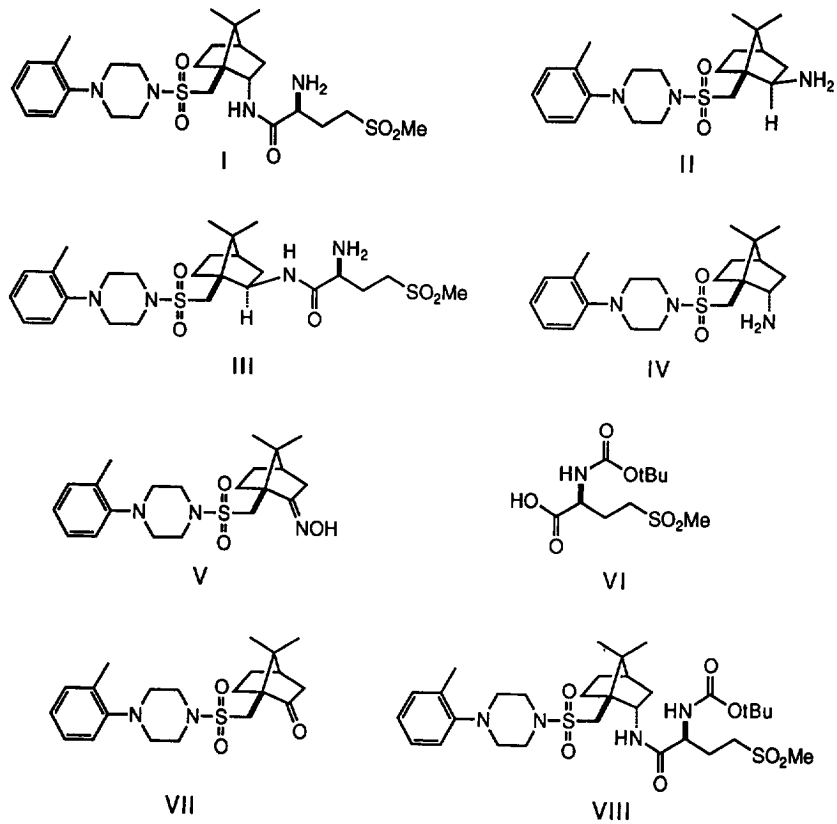

Fig. 1. Chemical structures of oxytocin antagonist and related compounds. (I) Oxytocin antagonist (L-368,899), (II) exo-amine (L-746,924), (III) exo-isomer of oxytocin antagonist (L-369,625), (IV) endo-amine (L-368,050), (V) oxime (L-367,433), (VI) N-boc-L-methionine sulfone, (VII) ketone (L-367,398), (VIII) boc protected oxytocin antagonist (L-368,845).

In this paper, an HPLC separation of an oxytocin antagonist, its exo-isomer, synthetic raw materials, and synthetic intermediates is reported. The structures of the oxytocin antagonist and synthetic process related compounds are shown in Fig. 1.

2. Experimental section

2.1. Solvents

All organic solvents were distilled in glass or of HPLC grade quality. Helium gas was used to deaerate the mobile phase. Filtered distilled water was treated with a Milli-Q System including a Millipore type HA 0.22-μm disk. The 0.005 *M* phosphate buffer (pH is approximately 6.4) was prepared by dissolving 0.20 g Na_2HPO_4 and 0.92 g KH_2PO_4 in 2 l water. The mobile phase was filtered through a 0.45-μm filter before use. The diluent used for the preparation of samples was a 50:50 mixture of 0.1 *M* phosphate buffer (pH 6.4) and acetonitrile. The concentration of solutions of compounds used for HPLC method development was approximately 0.5 mg/ml, with 20-μl injection.

2.2. Apparatus

Liquid chromatographic experiments were carried out with a Spectra-Physics (SP) Model SP8700XR liquid chromatographic pump complete with a SP Model AS3000 autosampler with a built-in column heater at 40°C and an Applied Biosystems 759A UV absorbance detector at 210 nm, with a Perkin–Elmer

GC–LC data system (Neslon Systems Inc., Cupertino, CA, USA). A 25 cm×4.6 mm I.D., Zorbax Rx C_8 column was used for the separation and during method development phases. The mobile-phase flow-rate was always between 1.0 and 1.5 ml/min.

3. Results and discussion

The eight components were chosen since they are either synthetic intermediates or key raw materials used in the process; therefore, the likelihood of finding these compounds as an impurity in the final product is significant. A water–acetonitrile (50:50) mixture was used initially as the mobile phase with reversed-phase (RP) columns for the isocratic separation of the eight-component mixture (Fig. 2A). Baseline resolution was achieved for compounds VII and VIII; however, compounds V, II, IV eluted as one band. The oxytocin antagonist (I), and its exo-isomer (III) were separated under these LC conditions, but they eluted as broad bands. Water was then replaced by 0.1% (v/v) phosphoric acid, providing an acidic mobile phase (Fig. 2B). Compounds I, II, III, IV and VI were not retained on the column and eluted in the void volume. All of these compounds, except compound VI, have a primary amino group, which is ionized at this acid mobile-phase pH, resulting in a decrease of the retention of these compounds. The retention times and selectivity of compounds V, VII and VIII were not significantly affected by the acidic conditions since they are uncharged. The results from acidic and neutral mobile phases (Fig. 2A and Fig. 2B) indicated better selectivity and retention under neutral or basic pH conditions. Therefore, 0.1% (v/v) triethylamine (TEA) was added to the 0.1% (v/v) phosphoric acid solvent. The chromatography achieved with this mobile phase revealed seven resolved bands (Fig. 2C); only the isomeric compounds II and IV were not resolved. Unfortunately, after several runs, the column exhibited an unaccountable loss of efficiency, in which compounds I and III were not always resolved. The irreproducibility was attributed to the lack of buffering capacity in the mobile phase.

The 0.1% TEA and 0.1% phosphoric acid in the mobile phase were then replaced by a pH 6.4

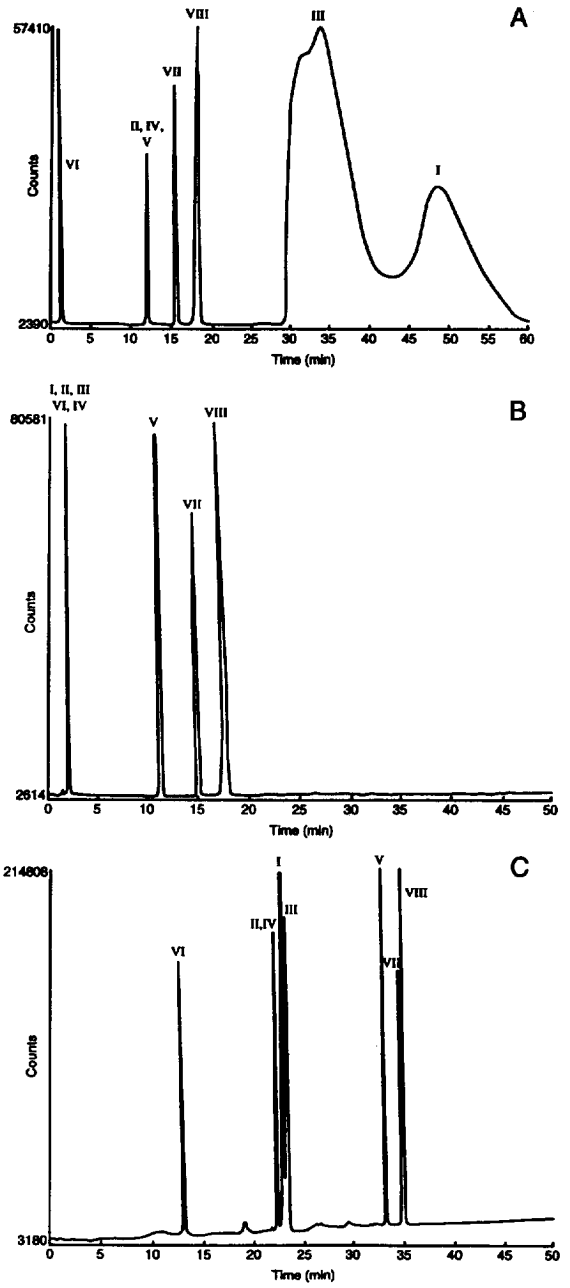

Fig. 2. (A) Chromatogram of oxytocin antagonist and related compounds test mixtures using a mobile phase of water–acetonitrile (50:50) under isocratic conditions. Labeled bands correspond to structures shown in Fig. 1. (B) Conditions the same as for (A), except 0.1% phosphoric acid in water is used instead of water in the mobile phase. (C) Using a two-component mobile phase: I, 0.1% phosphoric acid, 0.1% triethylamine in water; II, acetonitrile, and a linear gradient 95% solvent I to 95% solvent II over 50 min, 40°C, 1.5 ml/min, 210 nm.

phosphate buffer solution, which achieved retention with greater reproducibility (Fig. 3 and Fig. 4). The ionic strength of the phosphate buffer played a key role in the retention and selectivity of this eight-component mixture. At 0.02 *M* phosphate buffer concentration, compounds II and IV eluted as a single band and compounds I and III were not completely resolved (Fig. 3). At lower ionic strengths (0.01 *M* and lower), compounds I and III are better resolved and compounds II and IV were retained on column significantly longer. Under optimized mobile phase conditions, 0.005 *M* phosphate buffer–acetonitrile under gradient conditions (Fig. 4), all eight components were resolved. This optimized gradient condition gave baseline resolution of a pair of isomers (I, III) and (II, IV) and eluted the highly retained compound IV in a reasonable time. The change in ionic strength does not significantly change the retention of compounds V, VII and VIII. In this mobile phase, the basic compounds I, II, III and IV are partially protonated at pH 6.4, and probably interact with the column packing silanols by ion exchange. Increasing the salt concentration decreases ion-exchange interaction and increases solubility of the solute in the mobile phase, resulting in decreased retention of these basic compounds. Conversely, an increase in retention occurs when the salt concentration is decreased, resulting in decreased solubility in mobile phase. The effect of salt concentration and retention time change is more dramatic for compounds II and IV.

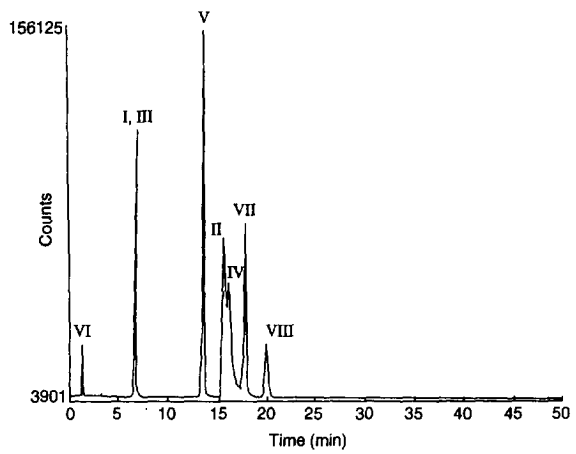

Fig. 3. Chromatogram of oxytocin antagonist and related compounds test mixture using a two-component mobile phase: 0.02 *M* phosphate buffer (pH 6.4)–acetonitrile (50:50), isocratic; flow-rate, 1.0 ml/min; all other conditions as in Fig. 2A.

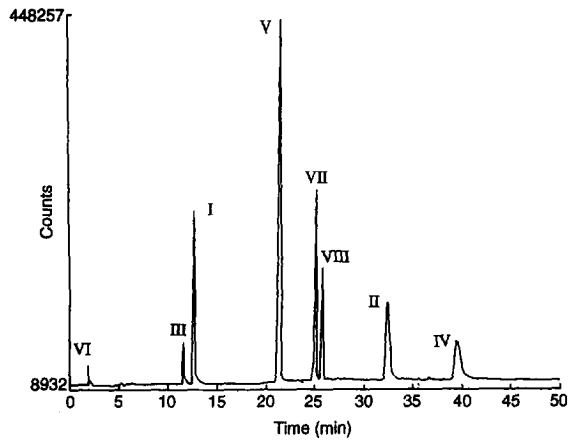

Fig. 4. Optimized chromatogram of oxytocin antagonist and related compounds test mixture using a two-component mobile phase: I, 0.005 *M* phosphate buffer (pH 6.4) in water; II, acetonitrile and a linear gradient as follows: *t* (min)=0, II=50%; *t*=12, II=50%; *t*=35, II=80%; *t*=50, II=80%; flow-rate, 1.0 ml/min; 30°C; 210 nm; all other conditions as in Fig. 2A.

The chromatographic method was further validated and the results are summarized in this paragraph. The HPLC area percent as a function of oxytocin antagonist (L-368,899) concentration showed linearity of the detector response of the L-368,899 peak over a range from 0.1 to 200% of the recommended assay concentration (0.5 mg/ml) with a correlation coefficient of 0.99997. The results from the linearity studies show that the lowest detectable level is less than 0.0005 mg/ml (0.1%). The signal-to-noise ratio at this concentration is greater than 10. This indicates that the method has a limit of detection of less than 0.1%. Method reproducibility was demonstrated by testing two solutions over a three-day period. The relative standard deviation observed for area percent, retention time, and response factor showed good day-to-day method reproducibility. The mean area percent was observed to be 99.03%±0.05% R.S.D., the mean retention time was observed to be 13.16 min±1.02% R.S.D., and the mean response factor (response factor=area

counts of main peak/concentration of main peak) was observed to be constant to within ±0.02% for three days. The method precision was tested by injecting five injections of a single assay preparation and measuring the peak area percent and peak retention times for both the L-368,899 and the exo-isomer (compound III). The method has good injection precision for L-368,899, with percent relative standard deviations of 0.12% (mean 13.01 min) and 0.05% (mean 99.03) for retention time and area percent, respectively. For the exo-isomer, the percent relative standard deviation of the retention time and area percent were 0.10% (mean 11.33 min) and 6.0% (mean 0.34), respectively. A Varian 9065 Polychrom diode-array detector was used to determine the homogeneity of the main peak. Spectra were taken at the front, apex, and tail of the L-368,899 peak. The spectra were qualitatively superimposable, indicating peak homogeneity.

4. Conclusion

A simple, sensitive, and precise HPLC method was developed to determine the purity of L-368,899. The method described allows the separation of L-368,899 and seven potential impurities. Several batches were evaluated using this method, none of these impurities were detected.

Acknowledgments

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