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LC determination of octreotide acetate in compound formulations of Sandostatin® and diamorphine hydrochloride

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Abstract

The determination of octreotide acetate in compound formulations of Sandostatin® and diamorphine hydrochloride by RP–LC is described. Octreotide acetate, diamorphine hydrochloride and their respective degradants, [des-Thr-ol^8]-octreotide and 6-*O*-acetylmorphine, were baseline resolved using a Lichrospher-60 RP-select B column with a mobile phase composition of acetonitrile/phosphate buffer (pH 7.4, 20 mM) (35:65 v/v) with UV detection at 210 nm. The method is simple, selective, precise and suitable for the determination of octreotide acetate in admixture.

Keywords: Octreotide acetate; Diamorphine hydrochloride; Degradants; Reversed-phase LC

1. Introduction

Octreotide acetate (Sandostatin®, Fig. 1), a long-acting octapeptide analogue of the hypothalamic hormone somatostatin is indicated for symptomatic control of metastatic carcinoid and vasoactive intestinal peptide-secreting tumours [1]. In palliative medicine, it is often necessary to administer octreotide acetate in combination with diamorphine hydrochloride [2], since co-administration simplifies treatment and improves the quality of life.

Octreotide acetate degrades to form primarily [des-Thr-ol^8]-octreotide [3], which is inactive. Diamorphine hydrochloride is unstable in aqueous solutions, hydrolysing to 6-*O*-acetyl morphine under the influence of pH, temperature and ionic strength [4]. Consequently, aqueous compound formulations of octreotide acetate and diamorphine hydrochloride may contain their respective

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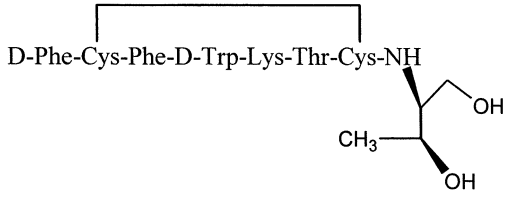
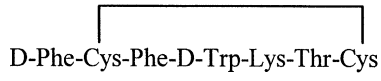
degradants. As nothing is known about the stability of octreotide acetate in the presence of diamorphine hydrochloride, suitable analytical methods are required which are selective for the determination of octreotide acetate in admixture.

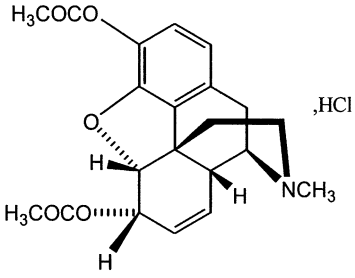
This study describes the development of a selective reversed-phase high performance liquid chromatographic method for the determination of octreotide acetate in the presence of diamorphine hydrochloride and their respective degradants, [des-Thr-ol^8]-octreotide and 6-*O*-acetylmorphine.



Octreotide



[Des-Thr-ol^8]-octreotide



Diamorphine hydrochloride

Fig. 1. Chemical structures of octreotide (Sandostatin®, SMS 201–995), [des-Thr-ol^8]-octreotide and diamorphine hydrochloride.

2. Experimental

2.1. Materials and reagents

Octreotide acetate, [des-Thr-ol^8]-octreotide and Sandostatin® ampoules (100 μg/ml and 500 μg/ml) were gifts from Novartis Pharmaceuticals (Surrey, UK). Diamorphine hydrochloride BP ampoules (100 mg) were obtained from Evans Pharmaceuticals, (UK). HPLC-grade acetonitrile, *ortho*-phosphoric acid (85%w/v), anhydrous sodium dihydrogen phosphate and anhydrous disodium hydrogen phosphate were obtained from BDH Laboratory Supplies (Poole, UK).

2.2. Sample preparation

2.2.1. Octreotide standard solutions

Standard solutions of octreotide acetate (25, 50, 75, 100 μg/ml) were prepared by suitable dilutions of a freshly constituted stock solution (5 mg/50 ml) with distilled water.

2.2.2. Octreotide acetate + diamorphine hydrochloride injection

Aliquots of Sandostatin® injection (4 ml, 100 μg/ml), Sandostatin® injection (1 ml, 500 μg/ml) and freshly constituted solution of diamorphine hydrochloride BP injection (0.5 ml, 100 mg/ml) were aseptically aspirated into a 10-ml polypropylene syringe. The content of the syringe was made up to 8 ml with water for injection BP to produce octreotide acetate (900 μg) + diamorphine hydrochloride (50 mg) per 8 ml of injection solution.

For HPLC analysis, this formulation was suitably diluted in water to produce solutions containing octreotide acetate at a nominal concentration of 50 μg/ml.

2.2.3. [Des-Thr-ol^8]-octreotide solution

[Des-Thr-ol^8]-octreotide (1 mg) was weighed into a 50-ml volumetric flask and made up to volume with distilled water.

2.2.4. Diamorphine hydrochloride solution

Diamorphine hydrochloride (5 mg) was weighed into a 100-ml volumetric flask and made up to volume with distilled water to produce a standard solution of concentration 5 mg/100 ml.

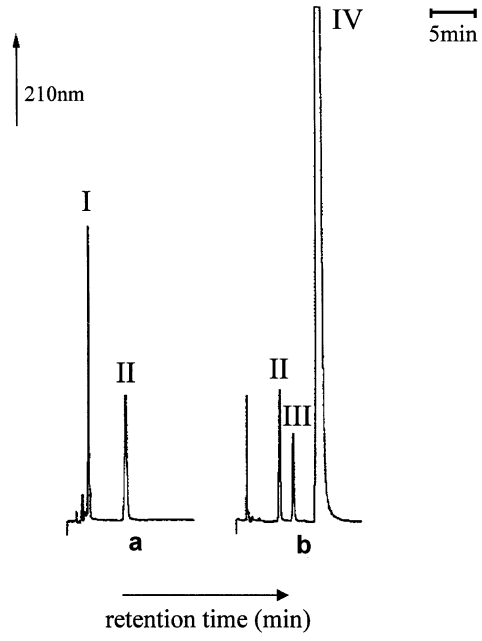


Fig. 2. HPLC chromatogram of (a) [des-Thr-ol[8]]-octreotide (**I**) and octreotide acetate (**II**) and (b) octreotide acetate (**II**), diamorphine degradant (6-*O*-acetylmorphine) (**III**) and diamorphine hydrochloride (**IV**) using a 125 × 4 mm Lichrospher® Select-B column (mobile phase: acetonitrile–phosphate buffer (pH 7.4, 20 mM) (35:65 v/v) with UV detection at 210 nm.

2.3. HPLC

The chromatographic system consisted of a Spectra–Physics P100 isocratic pump equipped with a Spectra–Physics SP8450 UV–vis detector (ThermoSeparations Inc., CA, USA) set at 210 nm with a response time of 0.5 s. Chromatograms were acquired and analysed with a HP3395 reporting integrator (Hewlett Packard, Waldbronn, Germany) with the following settings: attenuation, 8; peak-width detection, 0.04; threshold, 4. Chromatographic separations were performed at ambient temperature with a mobile phase flow rate of 1 ml/min. The mobile phase was filtered and degassed on-line using a 0.2-μm polypropylene Whatman solvent IFD (Whatman Scientific Ltd., Kent, UK). Samples were prepared in HPLC grade water and injected through a fixed (20 μl) PEEK loop onto a Lichrospher-60 RP Select B column (125 × 4 mm ID, 5 μm particle size, 60 Å) with a 20 × 2 mm ID guard column packed with pellicular C8 (30–40 μm particle size, Alltech Associates Inc., IL, USA). All standard and sample solutions were analysed in duplicate. The mobile phase was composed of acetonitrile–phosphate buffer (pH 7.4, 20 mM) (35:65 v/v). The phosphate buffer (Na_2HPO_4/NaH_2PO_4) was prepared by mixing 800 ml of 0.716% w/v Na_2HPO_4 and 190 ml of 0.276%w/v NaH_2PO_4 and the resulting solution adjusted to pH 7.4 with *ortho*-phosphoric acid.

3. Results and discussion

Octreotide acetate (t_R 5.5 min, **II**) was baseline separated from [des-Thr-ol[8]]-octreotide (t_R 2.0 min, **I**), diamorphine hydrochloride (t_R 11.4 min, **IV**) and the putative diamorphine degradant (t_R 8.1 min, **III**) using the HPLC conditions (Fig. 2a and b). Morphine hydrochloride (t_R 2.9 min), a degradant of diamorphine hydrochloride [4], was not detected in samples analysed within 24 h of preparation. The only secondary peak (**III**) in the standard solution of diamorphine hydrochloride, ascribed to 6-*O*-acetylmorphine [4], was observed to increase with time as a result of the hydrolysis of the parent compound. However, the separation between the peaks due to octreotide acetate (**II**) and the diamorphine degradant (**III**) was not compromised by the increase in the size of the latter with time. The robustness of the chromatographic conditions as measured by the variation (RSD) in retention times of the peak due to octreotide acetate over a 12-h period was 2.9% (5.6 ± 0.2 min, $n = 55$). Calibration solutions prepared within the range 0–100 μg/ml octreotide acetate and encompassing the nominal assay value of 50 μg/ml were linear ($R^2 = 0.999 \pm 0.002$, mean $\pm$ S.D., $n = 5$) with small standard residuals randomly distributed about the regression line. The regression equations were typically of the order $y = 2.4E5x + 8.1E4$. The precision of replicate injections of a 50 μg/ml solution of octreotide acetate was calculated as 99.02% ($n = 10$). The inter-assay precision (RSD) determined by replicate ($n = 6$) analyses of the same batch of Sandostatin® ampoules (500 μg/ml) was 0.93%.

The HPLC method was applied to the determination of octreotide acetate in a compound formulation of Sandostatin® and diamorphine hydrochloride (900 μg + 50 mg per 8 ml of solution). The content of octreotide acetate in the compound formulation was determined as 101.2 ± 1.0% (mean ± S.D., $n = 6$) of the nominal concentration.

4. Conclusion

The potential for interference of diamorphine hydrochloride (and its putative degradant, 6-*O*-acetylmorphine) and [des-Thr-ol[8]]-octreotide in the determination of octreotide acetate in admixture has been demonstrated. The chromatographic separation of octreotide acetate from these interferents outlined above provides the selectivity required for its determination in compound formulations containing diamorphine hydrochloride. The HPLC method described is simple, selective, precise and suitable for quality control and stability evaluation of compound formulations of octreotide acetate and diamorphine hydrochloride.

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