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Determination of apomorphine freebase in sublingual tablets by proton nuclear magnetic resonance spectroscopy

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ABSTRACT

An apomorphine sublingual tablet formulation under development contains mixtures of apomorphine freebase (FB) and apomorphine hydrochloride salt. It is important to have a reliable analytical method to determine the ratio of the base and salt forms to ensure accuracy, reproducibility and robustness of the manufacturing processes as well as to meet the requirements of the quality target product profile. A Proton Nuclear Magnetic Resonance (^{1}H NMR) spectroscopy method based on the proton shift of the amine methyl group (N-CH_3) in apomorphine has been developed to determine the mole percentage of freebase to the total mole of freebase and hydrochloride salt in the drug product. The method was evaluated in terms of specificity, linearity, and variability. The presence of excipients does not interfere with the analysis. A standard calibration curve of the chemical shift as a function of the proportion of freebase forms of apomorphine was established, covering the range of 100% apomorphine freebase to 100% apomorhine hydrochloride. The correlation coefficient (r^2), slope, and Y-intercept of the regression line are 0.998, −0.00596, and 3.191, respectively. The day-to-day variability of the ^{1}H shift in two instruments in the standard is less than 1% RSD. Three lots of the sublingual tablet drug product were examined and quantified by the standard. The mole percent apomorphine freebase was determined to be 73.8%, 75.2%, and 76.2%, respectively, within 100.0% ± 2.0% of the target value of 75.0%. The method is a new avenue to use the ^{1}H NMR technique for determination of apomorphine freebase and salt ratio in a solid drug product dosage form for release testing and in-process control.

1. Introduction

Apomorphine hydrochloride is a non-ergoline dopamine agonist and is chemically designated as 6αβ-Aporphine-10,11-diol hydrochloride hemihydrate with a molecular formula of $C_{17}H_{17}NO_2{\cdot}HCl{\cdot}0.5H_2O$ with molecular weight 312.79. The structure of apomorphine was determined in 1902 by Pschorr [1].

Its structural formula is shown in Fig. 1. The marketed drug Apokyn® is a sterile solution for subcutaneous injection as a treatment for Parkinson's disease (PD). The mechanism of action of apmorphine hydrochloride is believed to be due to stimulation of post-synaptic dopamine D2-type receptors within the caudate-putamen in the brain [2–6]. Multiple daily administrations of Apokyn® is an invasive procedure and troublesome for PD patients. Thus, there is a need for non-invasive routes of administration.

An apomorphine sublingual tablet under development at Impax Laboratories is intended for sublingual administration with rapid onset of action. The proprietary formulation is designed to dissolve the drug rapidly in the sublingual cavity to achieve rapid sublingual absorption, thereby circumventing the first-pass metabolism of oral administration. The permeability of apomorphine through sublingual tissue is enhanced by increasing the content of apomorphine base, providing a plasma profile similar to that of the subcutaneous injection product Apokyn. However, the freebase form of amorphonine is highly unstable, especially in an aqueous solution. The major degradation pathway is an oxidation, which forms apomorphine paraquinone or orthoquinone [7]. Therefore a mixture of the salt and freebase forms in a solid solution with an optimal ratio was developed reduce oxidation of apomorphine freebase to increase the stability of sublingual tablets and extend the shelf life of the drug product. Keeping the ratio within a consis-

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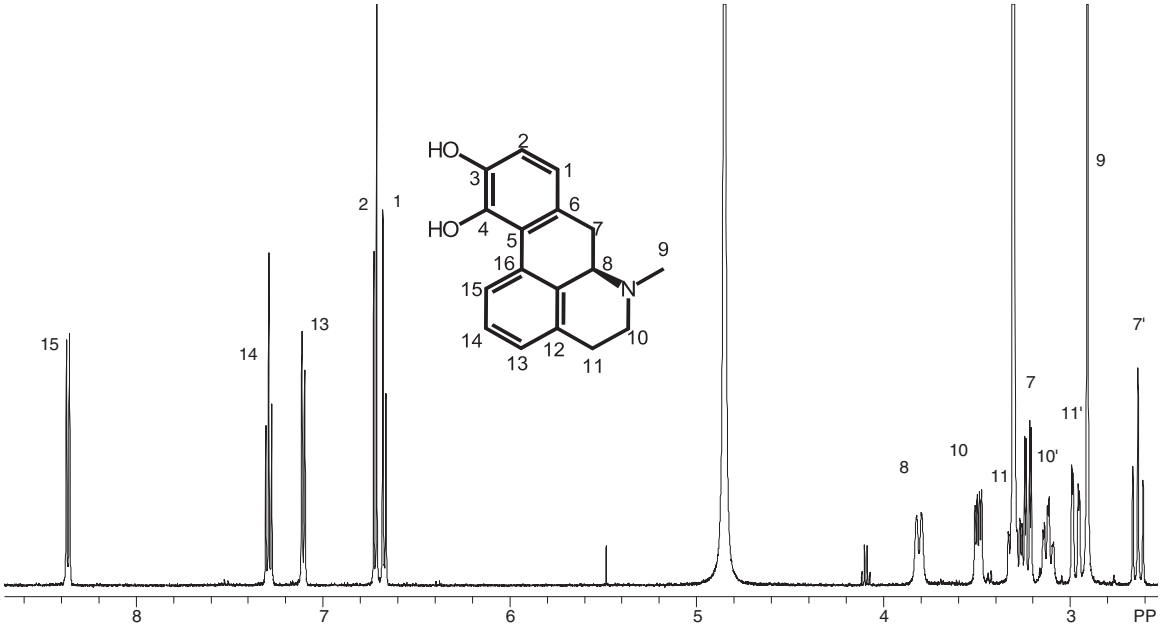

Fig. 1. Structure of and ^{1}H Assignment for Apomorphine. Solvent CD_3OD, standard concentration at total 5 mg/mL, 50% ApoFB (mole), the number 9 assigned to 3 protons on N-CH_3 group at 2.908 ppm.

tent range is critical as this may impact the final finished product quality and efficacy.

The titration assay method described in USP apomorphine monographs for drug substance and drug product [8] and published HPLC methods are intended to determine the total dose amount of apomorphine present in the drug substance, drug product or human plasma [9–11]. The USP method is based on titration of the amount of hydrochloride present. However, the apomorphine FB cannot be determined due to its lack of hydrochloride. The HPLC methods developed in-house for quantitation of apomorphine in the drug substance or drug product is based on the UV chromophore of apomorhine. After a sample is dissolved in the acidic preparation solution, all apomorphine from either source form is present as a non-associated, protonated form and remains in that state throughout the chromatographic separation process. Thus, the HPLC method cannot differentiate the apomorphine FB from its salt form. Since the drug product is a mixture of the freebase and the hydrochloride salt in sublingual tablets, it is essential to have a method which can determine these two different forms. One approach to solve this issue is to determine a combination of HPLC for total apomorphine amount and titration for apomorphine hydrochloride to calculate ratio of apomorphine freebase and apomorphine hydrochloride. Titrating hydrochloride in apomorphine hydrochloride includes using standardized reagents sodium bicarbonate and sulfuric acid solution, ether extraction and steam bath, which is tedious and lack of automation. Also, the method may be difficult to control with a precise end point based on a color indicator.

An NMR spectroscopy has been used to study ionization states of various molecules both in solution and in solid state [12–14]. Nuclear magnetic resonance (^{13}C NMR, ^{1}H NMR) techniques have long been used to determine equilibrium constants in amino carbamate analysis of carbamino adduction with carbon dioxide under physiological conditions [15–18]. The acid dissociation constants (pKa) in two propionic acid side chains in biliverdin and bilirubin have been determined based on chemical shifts in different pH solutions by ^{13}C NMR spectroscopy, which allows direct observation and quantitative measurement of the carboxylic acid and carboxylate anion carbon signals [19]. Over the last few decades, NMR has gained popularity for evaluation of the pKa and the quality of drugs, method validation, and the reference standard qualification [20–25].

In this study, the chemical shift of the amine methyl protons N-CH_3 in apomorphine is used as the ionization state indicator, where the magnitude of the shift will be affected by the ionization state due to the presence or absence of hydrochloride. The percent freebase of apomorphine present in the formulation can be quantified by the apomorphine freebase and apomorphine hydrochloride standard materials.

2. Experimental

2.1. Chemicals

Deuterated methanol-d4 (CD_3OD) containing tetramethylsilane (TMS) and dimethyl sulfoxide-d6 (DMSO-*d*6) were purchased from Cambridge Isotope Labs (Tewksbury, MA, USA). Apomorphine hydrochloride reference standard (RS, FW 312.8, lot I0M162) was purchased from USP (Rockville, MD, USA) and the purity was calculated based on the certificate of analysis. Apomorphine freebase reference standard (MW 267.3) was custom-synthesized by Bridge Organics (Vicksburg, MI, USA) with HPLC purity 98.6% and used as received. There is no correction for the purity. One lot of 5 mg (lot A) and two lots of 10 mg apomorphine tablets (lot B and lot C) and one lot of placebo tablet (lot D) were manufactured by Impax. Apomorphine freebase and apomorphine hydrochloride are abbreviated as ApoFB and ApoHCl, respectively.

2.2. Standard reference and sample solution preparation

The stock standard solution was prepared by dissolving 20.0 mg of ApoFB and 23.9 mg of ApoHCl in 0.7 mL of CD_3OD (62.7 mg/mL total apomorphine), resulting in a nominal 50% mole of ApoFB. The study was a range-finding quantitative analysis of the concentration effect. The solution was then diluted sequentially by factors of 4, 20 and 80 for evaluation of the concentration effect on ^{1}H shift.

For the calibration curve, different amounts of ApoFB and ApoHCl standards were weighed out and dissolved together in 10 mL