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ORIGINAL ARTICLE

HPTLC-densitometric method for simultaneous determination of salmeterol xinafoate and fluticasone propionate in dry powder inhalers

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Abstract A high performance thin layer chromatography (HPTLC) method was developed and validated for determination of two anti-asthmatic drugs, salmeterol xinafoate and fluticasone propionate in co-formulations. Study was performed on pre-coated silica gel HPTLC plates using *n*-hexane:ethyl acetate:acetic acid (5:10:0.2) as a mobile phase. A TLC scanner set at 250 nm was used for direct evaluation of the chromatograms in reflectance/absorbance mode. Method was validated according to ICH guidelines. Determination coefficients of calibration curves were found 0.9977 and 0.9936 in the ranges 100–1000 and 200–2000 ng band^{-1} for salmeterol and fluticasone, respectively. Method had an accuracy of 99.5% for salmeterol and 102.01% for fluticasone. Method had the potential to determine these drugs simultaneously from dosage forms without any interference.

1. Introduction

Salmeterol (Fig. 1a) is a long-acting and highly selective β_2 agonist formulated as its 1-hydroxy-2-napthoate (xinafoate) salt used in the treatment of asthma and chronic obstructive pulmonary disease (Michael et al., 2000; Murnane et al., 2006). Fluticasone propionate (Fig. 1b) is a neutral, highly potent trifluorinated corticosteroid based on the androstane nucleus. It is effective in treatments of asthma and allergic rhinitis because of its anti-inflammatory activity (Laugher et al., 1999; Krishnaswami et al., 2000). These two drugs are formulated as dry powder inhalers or pressurized metered dose inhalers individually or in combined formulation (Ringdal et al., 2007).

Validated assays have been reported for each drug individually. For analysis of salmeterol xinafoate from body matrices, liquid chromatography with MS (You-xuan et al., 2003),

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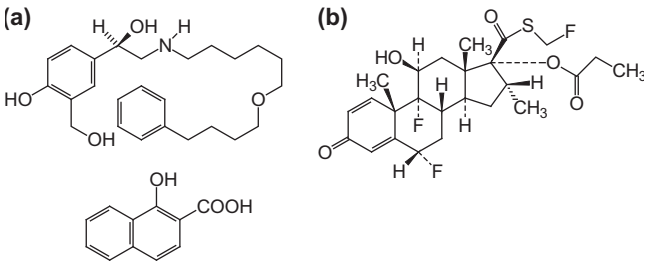

Figure 1 Chemical structures of salmeterol xinafoate (a) and fluticasone propionate (b).

MS/MS (Lehner et al., 2004) and fluorescence detection (Colthup et al., 2006) were reported. Spectrophotometric techniques have been reported for the determination of salmeterol xinafoate in its dosage forms (Chowdary and Rao, 1998, 1999; Reddy et al., 2000). Liquid chromatography coupled with APCI-MS (Yuan et al., 2001), and tandem mass spectrometers have been reported for the determination of fluticasone propionate in human plasma (Laugher et al., 1999; Krishnaswami et al., 2000; Vannecke et al., 2000).

There is a need for an assay method that permits simultaneous quantification of salmeterol xinafoate and fluticasone propionate. An HPLC method was reported for the concurrent analysis of these drugs in pressurized metered dose inhalers (Murnane et al., 2006). However, the detection in this method was done at 228 nm that is not free from interferences from solvents and other components. Moreover, the method was not validated for determination of these drugs in the powder for inhalers.

The aim of this work was to develop and validate a simple, rapid, selective and quite sensitive HPTLC assay method for simultaneous determination of salmeterol xinafoate and fluticasone propionate in bulk powders and dry powder inhalers. In addition, the method will be inexpensive and not requires certain types of stationary phases. Thus, it can represent another good alternative for the already existing HPLC methods especially that the detectors used for these methods are not present in most of the laboratories.

2. Experimental

2.1. Materials

Salmeterol xinafoate and fluticasone propionate working reference standards (Glaxowellcome, UK), analytical grade *n*-hexane (BDH, England), HPLC grade ethyl acetate (BDH, England), acetic acid (Fluka, Germany) and HPLC grade methanol (Sigma–Aldrich, Germany), were all obtained from Drug Administration and Control Authority of Ethiopia. The dosage forms (Seretide Accuhaler® 100/50, Seretide Accuhaler® 250/50 and Seretide Accuhaler® 500/50) were all purchased from retail out lets (Addis Ababa, Ethiopia).

2.2. Instrumentation

Microsyringe (Linomat syringe 659.004, Hamilton-Bonaduz schweiz, Camag, Switzerland), pre-coated silica gel 60 F-254 glass plates (10 × 10 cm with 200 μm, thickness HPTLC; Merck, Germany), linomat 5 applicator (Camag, Muttenz, Switzerland), twin trough chamber 20 × 10 cm (Camag, Muttenz, Switzerland), saturation pad (Camag, Muttenz, Switzerland), UV chamber (Camag, Muttenz, Switzerland), TLC scanner III (Camag, Muttenz, Switzerland), winCATS version 1.4.0 software (Camag, Muttenz, Switzerland) were used in this study. Microsoft excel was also used to treat data statistically.

2.3. Standard solutions

Stock standard solutions were prepared by dissolving 10 mg of salmeterol xinafoate and 20 mg of fluticasone propionate in 100 ml methanol to obtain concentration of 100 μg ml^{-1} and 200 μg ml^{-1} of salmeterol xinafoate and fluticasone propionate, respectively. Ten different concentration levels of working standard solutions were freshly prepared by diluting suitable volumes of the stock standard solution to 25 ml with methanol in appropriate volumetric flasks.

2.4. Sample solutions

2.4.1. Single-dose analysis

The contents of one blister were transferred quantitatively into 25 ml volumetric flask and the inner sides of the blister were washed with methanol three times. The volume was made up to about 20 ml with methanol and the contents were dissolved with the aid of shaking and sonication for about 10 min, then diluted to volume with the same solvent and filtered through 0.45 μm nylon syringe filters. Working sample solutions were freshly prepared by diluting suitable volumes of the stock sample solution to 25 ml with methanol in appropriate volumetric flasks.

2.4.2. Multi-dose analysis

The contents of five blisters were quantitatively transferred into 100 ml volumetric flask. The volume was made up to about 80 ml with methanol and the contents were dissolved with the aid of shaking and sonication for about 10 min, then diluted to volume with the same solvent and filtered through 0.45 μm nylon syringe filters. Working sample solutions were freshly prepared by diluting suitable volumes of the stock sample solution to 25 ml with methanol in appropriate volumetric flasks.

2.5. Chromatographic conditions

Sample was applied to the plate 10 mm from the bottom and 10 mm from the side edges in the form of band or streak with band length of 6 mm. The mobile phase consisted of *n*-hexane:ethyl acetate:acetic acid (5:10:0.2, v/v/v) and 15 ml of the mobile phase was used in each chromatographic run. Ascending development technique was carried out in a twin trough chamber. The optimized chamber saturation time for the mobile phase was 25 min at room temperature (20 ± 2 °C) that was assisted by saturation pad. The distance covered by the solvent front was 8 cm, which took about 15 min. The spots were scanned using the TLC scanner 3 in the reflectance/absorbance mode at 250 nm and all measurements were operated by winCATS software. Concentrations of the separated compounds were determined from the intensity of reflected light and peak areas were used for comparison.

2.6. Method validation

The method was validated in compliance with ICH guidelines (ICH 1994, 1996). The following parameters were used for validation of the developed method.