Chromatographic Methods for the Determination of Fluphenazine, Nortriptyline and Its Impurity Amitriptyline in Bulk and Pharmaceutical Formulations

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Received July 7, 2012; in final form, February 27, 2014

Abstract—Two selective, reproducible methods were proposed for the simultaneous determination of Fluphenazine hydrochloride (Flu) and Nortriptyline hydrochloride (Nor) in the presence of its impurity Amitriptyline hydrochloride (Ami). The first method is TLC-densitometric on silica gel with ethyl acetate–methanol–concentrated ammonia 2 : 7 : 0.2 (v/v/v) as mobile phase. The linear range 0.5–20, 0.5–5 and 0.5–5 μg per spot for Nor, Ami and Flu respectively. The second method is HPLC on Genesis C18 column with 0.1 M ammonium acetate–acetonitrile 1 : 1 (v/v) as mobile phase and Ultraviolet detection at 254 nm. The LC method was linear over concentration ranges 100–500, 50–300 and 10–250 μg/mL for Nor, Ami and Flu respectively. The proposed methods were successfully applied for determination of these drugs in laboratory prepared mixtures, and in pharmaceutical dosage forms showing good accuracy and precision. The results obtained agreed statistically with those obtained by using the official methods.

Keywords: nortriptyline, amitriptyline, fluphenazine, TLC-densitometry, HPLC

DOI: 10.1134/S1061934814120041

Nortriptyline Hydrochloride (Scheme) is a tricyclic antidepressant used in the treatment of depression, nocturnal enuresis, some anxiety disorders and neuropathic pain [1]. Nor is also known to be a metabolite of Amitriptyline [2]. Several methods were reported for its analysis such as LC–MS [3, 4], capillary electrophoresis [5] and spectrometric methods [6, 7].
Amitriptyline (Scheme) is a tricyclic antidepressant with the same pharmacological uses as Nor [1]. Ami is known to be one of the impurities of Nor, various methods were published for its determination such as LC–MS [8–10] and capillary electrophoresis [5].

Fluphenazine (Scheme) is a phenothiazine antipsychotic drug used to treat psychiatric disorders such as schizophrenia, mania, several anxiety and behavioral disturbances [1]. Several analytical methods were reported for its determination such as spectrometry [11], LC–MS [12–14], fluorescence spectrometry [15] and capillary electrophoresis [5, 16].

Nor and Flu are co-formulated in tablets, few methods were reported for their simultaneous determination such as LC–MS [4, 17–19], ratio derivative spectrometric methods [20]. Simultaneous determination of the Nor, Ami and Flu was done using capillary electrophoresis [5], and LC–MS methods [12, 14]. No TLC or simple LC with UV detection methods to our knowledge were reported for the simultaneous determination for the three drugs.

The scientific novelty in this work that it is the first TLC and LC methods proposed for the simultaneous determination of the three drugs. The proposed methods are suitable for the determination of the three drugs in routine analysis.

**EXPERIMENTAL**

**Chemicals, reagents, and solutions.** Nor and Flu standards of pharmaceutical grade were kindly supplied from Bristol Myers Squibb Pharmaceutical company, Cairo, Egypt. Their purities were checked according to the British Pharmacopoeia (BP) [19] and were found to be 99.9 ± 1.6 and 99.6 ± 0.8 for the two drugs respectively. Ami standard was kindly supplied from Al Kahira Pharmaceutical Company, Cairo, Egypt. Its purity was found to be 99.9 ± 1.0.

Pharmaceutical preparations: Motival tablets (batch number: B60317), labeled to contain 10 mg and 0.5 mg of Nor and Flu respectively; Tryptizol tablets (batch number: 12540), labeled to contain 25 mg Ami.

All solvents used were LC grade and all reagents were analytical grade. Ammonium acetate was obtained from Merck (Dramstadt, Germany). Methanol, concentrated ammonia (33%), ethyl acetate was from lab Scan Analytical Sciences (New Jersey, USA). Three stock solutions were freshly prepared before use: stock solution 1 containing 1 mg/mL Nor in methanol, stock solution 2 containing 1 mg/mL Flu in methanol and stock solution 3 containing 1 mg/mL Ami in methanol.

**Sample preparation.** Twenty Motival tablets were ground and thoroughly mixed. An amount of the powder equivalent to 100 mg Nor and 5 mg Flu was weighed and transferred to 100-mL volumetric flask, and 60 mL methanol was added. The solution was sonicated for 30 min (J.P. Selecta, Barcelona, Spain; CD 300513) then cooled. The solution was completed to volume with the same solvent, filtered (Whatman filter paper number 42) and the first 20 mL filtrate was discarded. The solution obtained was “sample solution”.

Twenty Tryptizole tablets were ground and thoroughly mixed. An amount of the powder equivalent to 100 mg Ami was weighed and transferred to 100-mL volumetric flask, continue to prepare sample solution for Ami similarly as Motival tablets sample solution was prepared.

**TLC densitometric method.** Procedure. TLC was performed on 20 × 20 cm aluminum foil plates coated with 0.2 mm layers of silica gel with fluorescent indicator (254 nm) (Fluka, Buchs, Switzerland). The plates were developed by ascending chromatography, to a distance of 16 cm with ethyl acetate–methanol–concentrated ammonia 2 : 7 : 0.2 (v/v/v) as mobile phase, in chromatographic tanks previously saturated for 1 h with mobile phase vapor. After development, spots were detected under a short-wavelength (254 nm) UV lamp, the plate was then scanned at 254 nm by means of Shimadzu (Tokyo, Japan) CS-9301 dual wavelength flying spot densitometer.

**Calibration.** Accurately weighed volumes of stock solution 1, 2 and 3 equivalent to 0.25–10 mg, 0.25–0.5 and 0.25–0.5 mg of Nor, Flu and Ami respectively, were transferred to three separate series of 5-mL volumetric flasks and the solutions were diluted to volume with methanol. Ten microlitres of each solution was applied using 10 μL pipette to TLC plates. Spots were separated 2 cm apart from each other and 1.5 cm from the bottom edge of the plate. A calibration plot for each drug was obtained by plotting peak area against drug concentration. Corresponding regression equations were calculated.

**Assay of laboratory-prepared Mixtures and Sample solution.** The procedure, described above, was repeated using sample volume equivalent to 0.8–6, 0.4–2.25 and 0.4–2.25 mg of Nor, Flu and Ami stock solutions respectively to comprise laboratory prepared mixture and extract obtained from sample preparation. The concentration of each drug was determined by using its corresponding regression equation.

**LC method.** Procedure. LC was performed with Hewlett Packard serried 1100, UV-Visible detector, and a manual injector with 20-μL loop. Compounds were separated on a Genesis column (5-μm, 250 mm × 4.6 mm i.d.), with 0.1 M ammonium acetate–acetonitrile 1 : 1 (v/v) at a flow rate of 1 mL/min. UV detection was at 254 nm, at ambient temperature.

**Calibration.** Accurately weighed volumes of stock solution 1, 2 and 3 equivalent to 1–5, 0.1–2.5 and 0.5–3 mg of Nor, Flu and Ami respectively, were transferred to three separate series of 10-mL volumetric flasks and the solutions were diluted to volume with methanol. Each solution (20 μL) was injected in triplicate. A calibration plot for each drug was obtained by plotting AUP against drug concentration. The number