Journal of Chromatography, 350 (1985) 273–278
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 080

SIMPLE THIN-LAYER CHROMATOGRAPHIC METHOD FOR THE INVESTIGATION OF SIX RELATED COMPOUNDS IN FLURAZEPAM HYDROCHLORIDE AND ITS CAPSULES

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(Received July 30th, 1985)

SUMMARY

A simple and rapid thin-layer chromatographic procedure is presented for the separation and tentative identification of flurazepam hydrochloride and its six related impurities in bulk samples and capsules. A methanolic sample solution is applied to a plate of silica gel containing a fluorescent indicator, which is developed once with a basic quaternary solvent system. Spots are visible under a short wavelength UV lamp, with a limit of detectability ranging from about 62.5 ng (related compound B) to 500 ng (related compound C). The proposed procedure shows several advantages over the related compounds test of the United States Pharmacopeia.

INTRODUCTION

Flurazepam hydrochloride is a pale yellow benzodiazepine compound whose hypnotic properties find use in the treatment of various types of insomnia[1]. The current literature cites up to six compounds as impurities of flurazepam hydrochloride[2–5]. These impurities, designated as related compounds A–F, enter flurazepam hydrochloride during the course of its synthesis[5,6] or following its degradation[7,8]. The USP XX[4] describes a thin-layer chromatographic (TLC) method for the identification of related compounds C and F. We found this method, which uses silica gel plates and a double development with fresh mixtures of diethyl ether–diethylamine, to be ineffective in resolving a mixture of flurazepam with its six related compounds. This objective can be accomplished with the TLC method presented here.

EXPERIMENTAL

Chromatographic studies were carried out on 5 × 20 cm and 20 × 20 cm precoated silica gel GF plates (0.25 mm thick, Analtech, Newark, DE, U.S.A.). The

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0021-9673/85/$03.30

developing solvent system consisted of diethyl ether–methylene chloride–diethylamine–triethylamine (90:10:2:1, v/v/v), prepared fresh just prior to use from analytical reagent grade solvents and amines. Samples were applied to the plates by means of 10-μl disposable micropipettes (Microcaps®, Drummond Scientific, Broomall, PA, U.S.A.). Plates were developed in filter paper-lined, pre-equilibrated glass tanks. The spots were located by viewing the plates under short-wavelength and long-wavelength UV lights. The sample of flurazepam was USP Reference Standard (U.S. Pharmacopeial Convention, Rockville, MD, U.S.A.) and related compounds A–F were a generous gift (Hoffman-La Roche, Manati, PR, U.S.A.). Standard solutions of flurazepam hydrochloride (100 000 μg/ml), related compounds A–D and F (100 μg/ml), and related compound E (200 μg/ml) were prepared in methanol. The solutions are stable for several weeks if kept away from light.

For the analysis of suspect samples, the content of one capsule, or a suitable quantity of drug substance, was dissolved in methanol to give a solution containing about 0.1% of flurazepam hydrochloride.

Solutions of the standard and samples were applied to a TLC plate at 1.5–2.0 cm intervals and 2.5 cm away from one of the edges of the plate. The plate was allowed to air-dry at ambient temperature and then was developed with developing solvent in a chromatographic tank, to a minimum distance of about 12 cm from the origin. After marking the solvent front, the plates were removed from the chromatographic tank, allowed to air-dry inside a hood, and sequentially examined under short- and long-wavelength UV lights.

RESULTS

The chemical names and structural formulae of flurazepam hydrochloride and its related compounds are given in Table I. The corresponding hR_F ($= R_F \times 100$) values are listed in Table II together with the limits of detectability under short-wavelength UV light. Only related compound B was visible under long-wavelength UV light.

Fig. 1 shows representative TLC separations of synthetic and contaminated commercial samples of flurazepam hydrochloride. A lot of flurazepam hydrochloride capsules, suspected of being degraded, was analyzed by the manufacturer's TLC identification method for flurazepam hydrochloride, and found to contain three spots other than flurazepam, one of which was identified as related compound C. The proposed TLC method, on the other hand, disclosed four spots with migrating characteristics that matched those of related compounds A, C, D, and E.

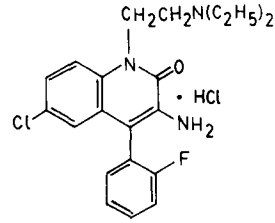
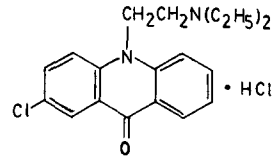
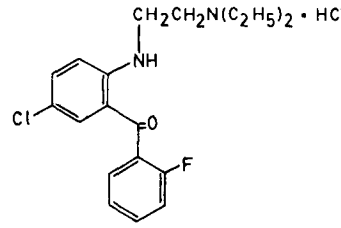
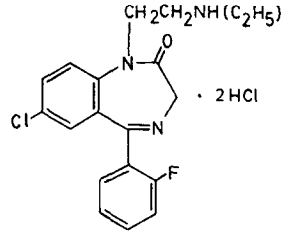
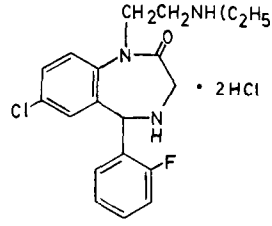
A mixed standards solution in methanol yielded an additional spot upon storage for several weeks. This spot was located just below related compound C (Fig. 1).

DISCUSSION

Related compound A is an aminoacetamide intermediate for the synthesis of flurazepam[8]. Related compound B is a breakdown product formed when an aqueous solution of flurazepam is exposed to an alkaline environment[7], whereas related compound C can be formed under alkaline and acidic conditions[7]. Related compound E is a degradation product of flurazepam[6] which upon exposure to air will readily

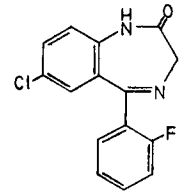
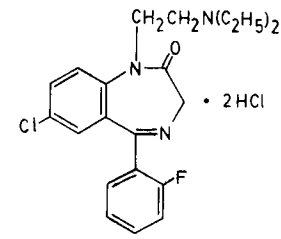
TABLE I

CHEMICAL NAMES AND FORMULAE OF FLURAZEPAM HYDROCHLORIDE AND ITS RELATED COMPOUNDS

Related compound	*Chemical name*	*Formula*
A	3-Amino-6-chloro-1-[2-(diethylamino)-ethyl]-4-(2-fluorophenyl) carbostyril hydrochloride	
B	2-Chloro-10-[2-(diethylamino)ethyl]-9-acridanone hydrochloride	
C	5-Chloro-2-[2-(diethylamino)ethylamino]-2'-fluorobenzophenone hydrochloride	
D	7-Chloro-1,3-dihydro-1-[(2-ethylamino)-ethyl]-5-(2-fluorophenyl)-2H-1,4-benzo-diazepin-2-one dihydrochloride	
E	7-Chloro-1-[2-(ethylamino)ethyl]-5-(2-fluorophenyl)-1,3,4,5-tetrahydro-2H-1,4-benzodiazepin-2-one dihydrochloride	

(Continued on p. 276)

TABLE I *(continued)*

Related compound	*Chemical name*	*Formula*
F	7-Chloro-5-(2-fluorophenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one	
Flurazepam hydrochloride	7-chloro-1-[2-(diethylamino)ethyl]-5-(2-fluorophenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one dihydrochloride	

become oxidized to related compound D[6]. Related compound F is the main degradation product of flurazepam in aqueous solution[7]. The latter compound together with related compounds B, C, and F are also known metabolic products of flurazepam in man[9–11].

Although related compounds A, B, D, and E are listed in the *Pharmacopeial Forum*[3], the official compendia only requires purity tests for related compounds C and F[2,4]. That the remaining impurities may also be found in commercial samples is demonstrated by the results that we obtained on a lot of flurazepam hydrochloride capsules that had been collected by Food and Drug Administration inspectors during routine surveillance work. Despite the lack of published information on the toxicological significance of these impurities, we are currently developing procedures that will verify the levels at which they occur in commercial samples so that an estimate can be made on their contribution to the labeled amount of active ingredient.

Various problems were encountered with the compendial TLC procedure. Firstly, it required three changes of fresh developing solvent for one TLC test. Secondly, related compound C tended to migrate near the solvent front. Thirdly, related compound B was not resolved from flurazepam. The recommended solvent system consists of diethyl ether–diethylamine (150:4, v/v), which is approximately equivalent to a 2.67% solution of the amine in diethyl ether. Experiments using concentrations of amine between 1–2% caused the spot of flurazepam to tail. This problem was obviated by raising the concentration to 3% or greater, but with the loss in resolution among those spots migrating ahead of flurazepam. Substituting triethylamine for diethylamine resulted in well rounded, compact spots, but with some spots showing little migration. This problem was more evident as the concentration of triethylamine was raised from 1 to 3%. In view of these results, it was

TABLE II

hR_F (= R_F × 100) VALUES AND DETECTION LIMITS OF FLURAZEPAM HYDROCHLORIDE RELATED COMPOUNDS

Related compound	hR_F*	*Limit of detection (ng)*	*UV detection mode (nm)*	
			254	*365*
A	71.0	250	+	–
B	64.5	62.5	+**	+**
C	83.5	500	+	–
D	26.0	125	+	–
E	30.5	125	+	–
F	43.5	125	+	–
Flurazepam hydrochloride	60.5	–	+	–

* The average of three chromatograms on silica gel GF layers developed with diethyl ether–methylene chloride–diethylamine–triethylamine (90:10:2:1, v/v/v).

** Blue fluorescence.

decided to adopt a solvent system that will incorporate both amines, namely one containing diethyl ether–diethylamine–triethylamine (187:2:1, v/v/v). Since related compound B still overlapped with flurazepam, it was found necessary to add a certain proportion of methylene chloride to effect their resolution. In this respect, chloroform was unsuited because of its content of ethanol which tended to raise the R_F values excessively. Also, since the official compendia does not specify the type of diethyl ether to use in the preparation of the solvent system, we decided to compare the use of the anhydrous (absolute) grade against the non-anhydrous (reagent) grade. The latter grade yielded better separations, particularly among related compounds B, D, and E. The differences are ascribed to the presence of ethanol, added as a preserv-

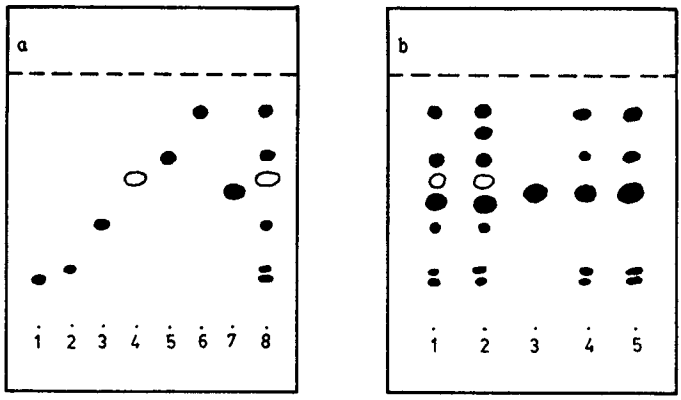


Fig. 1. TLC separation of flurazepam hydrochloride related compounds. (a) Standard related compounds: 1, D; 2, E; 3, F; 4, B; 5, A; 6, C; 7, flurazepam hydrochloride; 8, mixture of 1–6. (b) 1, mixture of standard related compounds and flurazepam hydrochloride, fresh solution; 2, mixture of standard related compounds and flurazepam hydrochloride, old solution; 3, flurazepam hydrochloride; 4 and 5, flurazepam hydrochloride capsule suspected of being degraded. All spots were detected under short-wavelength UV light.

ative, and possibly to traces of water in the reagent grade diethyl ether. None of the solvent systems described by De Silva and Strojny[11] for the TLC separation of related compounds B, C, and F in urine samples yielded the results required in the present study.

Pre-equilibration of the chromatographic tank with the solvent system precluded the formation of a concave solvent front, which otherwise would have caused the distortion of the chromatogram.

At the levels of impurities tested, all of the spray reagents tested failed to yield discernible spots. Fortunately, flurazepam and its six related compounds were clearly visible as quenching spots under a short-wavelength UV light. An additional identifying characteristic for related compound B was its blue fluorescence under long-wavelength UV light[9].

In summary, the TLC method described in this paper will simultaneously separate flurazepam from related compounds A–F using a single development with a quaternary solvent system. Satisfactory detection of the impurities is achieved by viewing the plates under short-wavelength UV light, to a minimum concentration of between 62.5–500 ng, depending on the impurity.

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