High Performance Thin-Layer Chromatographic Method for the Quality Control and Stability Assay of Hexoprenaline

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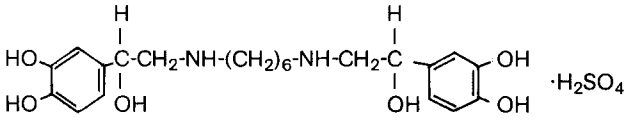
1 Introduction

Hexoprenaline (*N,N*-bis[2(3′,4-dihydroxyphenyl)-2-hydroxyethyl]hexamethylenediamine) sulfate is a catecholamine-like betamimetic with excellent bronchiolytic properties [1]. Chemically it contains two molecules of norepinephrine linked together via their nitrogen atoms by an hexamethyl group, as illustrated below



Clinical studies have shown that it is indicated in obstructive pathologies of the respiratory tract [2]. Also, compared to other related drugs, such as isoproterenol and rimiterol, its pharmacological effect is more persistent.

Though this drug is presently marketed by several pharmaceutical companies, to our knowledge, apart from the initial pharmacokinetic studies carried out with the tritium labeled analogue [1], no practical assay has been reported for its separation and quantitation in pharmaceutical formulations. Thus, for the purpose of drug stability studies and rapid quality control determinations a simple assay procedure has been developed for the low level detection of hexoprenaline sulfate in the presence of the array of excipients usually contained in commercial products. As hexoprenaline is rather labile and not readily amenable to gas chromatographic analysis, and taking into account that its parent substance, norepinephrine, has been detected in trace levels by fluorescence derivatization [3,4], the method involves the HPTLC fluorescence quantitation of the dansyl derivative of hexoprenaline down to the low picogram level. The specificity is such that no preliminary sample clean up is needed and the reaction mixture can be directly applied to the HPTLC plates.

2 Experimental

The acetone, sodium bicarbonate, benzene, methanol, and triethylamine used in this work were all a.g. from the local Merck supplier. The 5-dimethylamino-1-naphthalenesulfonyl chloride (Dansyl. Cl) was also from Merck.

2.1 Analytical Procedure

A 3 ml aliquot of the sample to be derivatized (aqueous solution of injectable product at pH 2.9, containing 2.5 μg/ml hexoprenaline) was placed in a screw-capped opalescent tube with 60 μl of an aqueous solution of EDTA Na_2(12 μg/ml) and 1 ml of a solution of Dansyl-Cl in acetone (1 mg/ml). The tube was tightly closed, shaken, and reopened to introduce 500 mg of sodium bicarbonate. For complete solution, the mixture was vigorously shaken for 30 s and placed in an oven at 35°C for 30 min. After cooling to room temperature, 1 ml of benzene was added with strong agitation for 2 min. The contents of the tube were then centrifuged at 3000 rpm for 2 min, and 0.5 ml of the supernatant benzene extract subsequently transferred to a minivial with subsequent evaporation to dryness under a steam of purified nitrogen. The residue was redissolved in 0.5 ml of benzene. Aliquots (2 μl) of this solutions were directly spotted on the HPTLC plates.

The time course of these reactions was followed at the preestablished optimum reaction temperature of 35°C. According to the experimental data, the reactions are practically complete in 15 min; however, for better reproducibility the reaction time was standarized to 30 min. The influence of the pH of the reaction mixture on the yield of the derivative is illustrated by the marked bell shaped response curve obtained between pH 8-10 with the maximum response at pH 9 and minima at pH values of 8 and 10.

The samples containing hexoprenaline were spotted on Merck plates precoated with Silica Gel $60F_{254}$ (10x20 cm) for nano-TLC with concentration zone. Volumes of 2 μl of the samples diluted in benzene to a concentration of around 7.5 μg/ml were applied with an Evachrom sample applicator. Under these conditions the amount of hexoprenaline applied to the plates is of the order of 15 ng. The HPTLC plates were developed in the dark with a solvent system of benzene/triethylamine (8:1). The front migration distance was 4 cm and the development time around 7 min. In this system the dansyl derivative of hexoprenaline gives an R_f value of 0.28.

Reverse phase HPTLC of this derivative was also carried out on Merck precoated RP-18 plates (10 x 10 cm) using the same application system. These plates were developed with methanol/water (93:7) during 16 min, giving an R_f value of 0.13 with a migration distance also of 4 cm.

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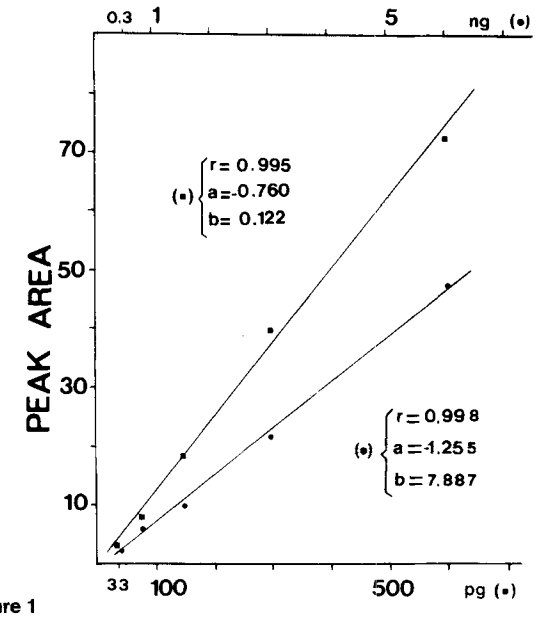

Figure 1

Linear response from the fluorescent dansyl derivative of hexoprenaline in two HPTLC systems.
(●) RP-18 plates and samples applied with a 2 µl microcapillary.
(■) SiO_2 plates and samples applied with a 0.2 µl microcapillary.

Table 1

Reproducibility of the method.

	Hexoprenaline standard	Reconstituted samples		Commercial formulations	
		A	B	A_1	A_2
$\bar{x}$ [µg]	2.5	2.5	2.3	2.4	2.4
s	0.05	0.07	0.09	0.09	0.09
Sr [%]	2.1	2.7	3.8	3.6	3.6

n = 5 individual derivatization reactions of which aliquots of 2 µl each were spotted on the HPTLC plates and assayed according to the experimental procedure using the data pair technique [5] for quantitation.

Table 2

Recovery and precision in the assay of hexoprenaline in different pharmaceutical forms.

	Batches				
	A	B	C	D	E
$\bar{x}$	96.7	100.1	101.8	99.9	96.3
n	4	9	4	7	4
Sr [%]	5.1	6.1	3.3	5.5	6.1

A, batch used in the manufacture of compressed tablets (0.5 mg/tablet); B through E, batches used in injectable solutions (5 µg/injectable).
$\bar{x}$, values of n determinations carried out within 30 days of manufacture date given in percentage of declared label value.
The assay of compressed tablets requires extraction of 1 tablet into 150 ml of a H_2SO_4 solution at pH 2.9, prior to the derivatization of a 3 ml aliquot as described in the text for injectables.

The in situ quantitative HPTLC measurements reported herein were performed with a Zeiss KM3 chromatogram spectrophotometer operating in the fluorescence mode. The monochromator was set at a λ_{max} of 313 nm for excitation using the FL46 filter for emission. The slits were set at 3.5 x 0.5 mm and the hexoprenaline spots were scanned at a speed of 120 mm/min with a recording paper speed of 120 mm/min. Peak areas were computed with the aid of an Minigrator electronic digital integrator from Spectra Physics.

3 Results

3.1 Detectability Limits and Calibration Curves

The response of the hexoprenaline derivative relative to the absolute amount deposited on the plate was established in two different HPTLC systems. On silica gel plates it was possible to reach a level of 33 pg of hexoprenaline base (80 femtomoles), as illustrated in **Figure 1**. This was accomplished on use of sample application capillaries of 0.2 µl. The use of 2 µl capillaries only allowed detectability limits of the order 150 pg (350 femtomoles).

On reverse phase RP-18 HPTLC plates and with 0.2 µl capillaries the minimum amount detectable was 150 pg; the corresponding amount was about 300 pg on using the 2 µl microcapillaries (Figure 1).

3.2 Application of the Method to Reconstituted and Commercial Formulas

The overall reproducibility of the reaction of hexoprenaline with Dansyl.Cl and subsequent spectrophotodensitometric determination of the derivative was checked by replicate determinations of a standard, a reconstituted sample (prepared by mixing in the laboratory all of the formulation ingredients), and a recently manufactured commercial batch. The results are given in **Table 1**. As indicated, the Sr for replicate determination is <4%. The method has been applied to the routine control assay of the declared content of hexoprenaline in various commercial preparations, giving the results shown in **Table 2**.

An acid-base potentiometric continuous titration with 0.02 N NaOH of the hydrochloric acid released by the dansylation process [3] indicates that hexoprenaline takes up four Dansyl groups. Also, this process is remarkably enhanced by the addition of a relatively large excess of EDTA with the response, expressed as peak area, increasing by up to 30% when the derivatization is carried out in the presence of 700 µg of EDTA.

Finally, the possibility of degradation of hexoprenaline into either epinephrine or norepinephrine during the experimental procedure was ruled out by the absence of peaks at the R_f values corresponding to their Dansyl derivatives on the SiO_2 plates (0.28, 0.44, and 0.14, respectively).

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