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Note

Determination by high-performance liquid chromatography of clenbuterol in commercial syrup formulations

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Clenbuterol (4-amino-3,5-dichloro-α-*tert.*-butylaminomethylbenzyl alcohol hydrochloride) is a beta-adrenergic drug used as a bronchial dilating agent. It is recommended for the treatment of asthmatic disease and, due to its long-term high activity, it is used especially in the case of chronic illness[1–7]. Beacuse of its antidepressive like activity in animals, it is also suggested for human endogenous depression[8].

Various methods for the assay of clenbuterol in biological samples have been reported. Radiological [9,10], enzymatic[11] and chromatographic methods [thin-layer chromatography[12], gas chromatography[13,14], high-performance liquid chromatography (HPLC)[15–17]] have been used for pharmacokinetic studies and for routine analysis in racehorses. Moreover, an HPLC method was developed for pharmaceutical gel formulations[18]. As part of our research on clenbuterol pharmaceutical preparations [19,20], we now describe a rapid and easy to reproduce procedure which allows an assay of clenbuterol in syrups, directly on the sample itself, without any previous extraction of the drug. In this way, possible recovery problems are avoided. Direct injection of the sample appears particularly opportune for an adequate and reliable quality control of these preparations which contain a very small quantity of an highly active drug in the presence of the large number of excipients usually contained in the syrups (1–2 μg clenbuterol per ml syrup).
This method is applicable to all the commercial formulations available in Italy, even when the excipients are different and numerous. It consists in a modification of the method proposed by Hamann *et al.*[18] for gel formulations.

EXPERIMENTAL

Materials

Clenbuterol was provided by Resfar (Milan, Italy). HPLC grade methanol and isopropanol were from Carlo Erba (Milan, Italy) and heptanesulphonic acid sodium salt monohydrate was from Fluka (Buchs, Switzerland). All solutions and solvents were filtered through a Millipore filter, pore size 0.45 μm (Millipore, Bedford, MA, U.S.A.).

HPLC instrumentation

Analytical liquid chromatography was performed using a Waters HPLC appa-

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ratus (Waters Assoc., Milford, MA, U.S.A.) consisting of a Model 510 Pump, equipped with an U6K injector, a variable-wavelength UV detector (Waters 450). Chromatographic data were collected and processed with a LC 100 Perkin-Elmer integrator (Norwalk, CT, U.S.A.). A stainless-steel column (25 cm × 4.6 mm I.D.) was packed with 7-μm CN stationary phase (LiChrosorb CN; Merck, Darmstadt, F.R.G.).

Operating conditions

The following chromatographic conditions were used. Mobile phase: water–methanol–isopropanol (70:29:1, v/v) containing 0.10% (w/v) heptanesulphonic acid sodium salt, degassed before use. Flow-rate: 1 ml/min. Column temperature: ambient. Volume injected: 100 μl. Detector wavelength: 246 mm. Detector sensitivity: 0.01 a.u.f.s. The column was carefully washed every day with 30 ml water and 30 ml methanol.

Linearity of detector response vs. standard concentration

A calibration graph was obtained by injecting clenbuterol solution in a concentration range of 0.5–10 μg/ml.

Sample preparation

The syrup samples (100 μl) were injected as such without any purification or dilution.

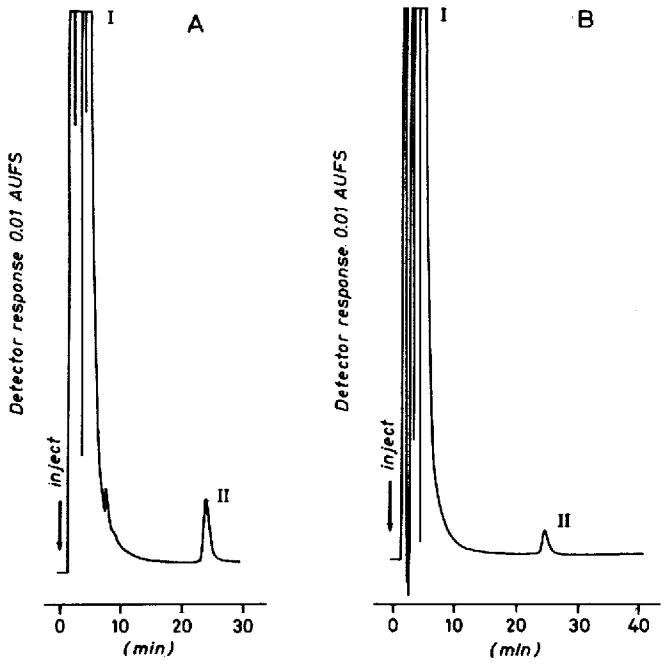

Fig. 1. Chromatograms of two different commercial clenbuterol syrups (A, 2 μg/ml; B, 1 μ/ml). Peaks: I = excipients; II = clenbuterol.

TABLE I

DAY-TO-DAY REPRODUCIBILITY OF THE CALIBRATION GRAPH FOR CLENBUTEROL

Day	*Slope*	*Correlation coefficient*
1	1698.6	0.9953
2	1716.0	0.9996
3	1739.6	0.9997
Mean	1718.1	
S.D.	20.6	
C.V. (%)	1.2	

RESULTS AND DISCUSSION

Our modifications of the Hamann method[18] permitted a good separation between clenbuterol and the other components of all the pharmaceutical syrup formulations commercially available in Italy, without any purification of the sample. Fig. 1 shows the chromatograms of two (A, B) of the seven syrups analyzed. For all the other formulations examined, a comparably good separation of the clenbuterol from the excipients was obtained.

The calibration graph showed good linearity with a correlation coefficient of 0.9997: $y = 1739.6x - 0.277$.

A day-to-day reproducibility test was performed over 3 days. A good reproducibility of the slope of the calibration graph was obtained (coefficient of variation, C.V. = 1.2%). The slopes and the linear correlation coefficients are reported in Table I.

The detection limit was approximately 0.1 ng, calculated on a response of twice the noise level.

The reproducibility of the determination of clenbuterol in syrups was satis-

TABLE II

REPRODUCIBILITY FOR DETERMINATION OF CLENBUTEROL IN A SYRUP

Injection	*Concentration (μg/ml)*
1	1.08
2	1.02
3	1.01
4	1.01
5	1.07
6	1.15
7	1.09
8	1.09
Mean	1.06
S.D.	0.05
C.V. (%)	4.72

TABLE III
RECOVERY STUDY

Placebo	*Clenbuterol added (μg/ml)*	*Recovery (% ± S.D.)*
A	1.08	96.8 ± 2.3
B	1.00	98.2 ± 2.0
C	1.94	99.0 ± 2.1
D	1.85	98.0 ± 2.2

factory, as shown in Table II which reports the response to repeated injections of one of the syrups analyzed (C.V. 4.72%).

Four different placebo syrups were prepared according to some commercial formulations. No interfering peaks were present at the retention time of clenbuterol for all these placebos. Known amounts of clenbuterol (1–2 μg/ml) were added and recovery experiments were carried out (Table III).

The column was carefully washed every day, as mentioned. In this way, no efficiency loss was observed throughout our work.

In conclusion, this HPLC method for the determination of clenbuterol in syrups appears to be reproducible and sensitive. In addition, it allows clenbuterol to be determined in these pharmaceutical formulations without any sample preparation, making this procedure very easy to perform. Finally, it provides a reliable quality control of these preparations.

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