Journal of Chromatography, 231 (1982) 210—215
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 1304

Note

Determination of tolazoline in plasma by high-performance liquid chromatography

V. ROVEI*, G. REMONES, J.P. THENOT* and P.L. MORSELLI

Department of Clinical Research, LERS - Synthelabo, 58 Rue de la Glacière, 75013 Paris (France)

(Received February 24th, 1982)

Tolazoline is an alpha-adrenergic blocking agent which also exhibits dilator action on the peripheral blood vessels. For this reason, it is recommended in the treatment of peripheral vascular disorders such as acrocyanosis and Raynaud's syndrome [1, 2]. Tolazoline has also been shown to be a histaminergic agonist acting at H_2 receptors [3]. Recently, tolazoline has been proposed as a vasodilator for newborns suffering from pulmonary hypertension. As small plasma volumes (< 0.2 ml) are available in these patients, a sensitive method is required for monitoring drug concentrations.

Several methods have been described for measuring tolazoline and other imidazoline derivatives [4—8]. These methods are generally based on spectrophotometry, colorimetry, thin-layer chromatography, gas—liquid chromatography and liquid column chromatography.

The spectrophotometric and colorimetric techniques are often not specific because they cannot differentiate the parent compound from the metabolites or the degradation products. Thin-layer and gas chromatography require several steps for the analysis of these compounds and are therefore time consuming.

Liquid column chromatography appears to be a suitable technique, which allows the analysis of the compound underivatized straight after extraction from biological fluids. We describe in this paper a high-performance liquid chromatographic (HPLC) method suitable for the monitoring of tolazoline plasma concentrations in newborns.

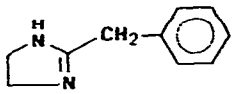
*Present address: Laboratoire Delalande, 92500 Rueil-Malmaison, France.

0378-4347/82/0000—0000/$02.75

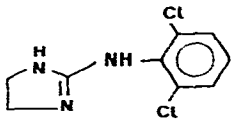
EXPERIMENTAL

Standard and reagents

Tolazoline hydrochloride [4,5-dihydro-2-(phenylmethyl)-1H-imidazole hydrochloride] was supplied by Dr. Monin, Maternité Universitaire of Nancy, France. Clonidine hydrochloride [2-(2,6-dichlorophenylamino)-2-imidazoline hydrochloride], the internal standard, was supplied by Dr. Cavero, Department of Biology, Synthélabo-L.E.R.S., Paris, France. Both compounds were checked for purity by mass spectrometry.



TOLAZOLINE



CLONIDINE

Aqueous solutions of tolazoline (1 mg/ml, 100 μg/ml and 10 μg/ml) and clonidine (100 and 10 μg/ml) were prepared. The final concentrations were calculated as free bases for both compounds. Thes solutions were found to be stable for 3—4 months at 4°C.

Analytical-reagent grade chloroform and acetonitrile (Uvasol) were purchased from Merck (Darmstadt, G.F.R.).

Extraction procedure

A 20-μl volume of an ethanolic solution of clonidine (10 μg/ml) was added to a tapered conical tube together with 0.5 ml of 0.25 *M* potassium hydroxide solution and 0.2 ml of plasma sample. This solution was extracted with chloroform (7 ml) on a shaker (Bioblock) for 20 min.

The sample was centrifuged for 5 min at 1000 *g* and 4°C and the upper aqueous phase was discarded. Approximately 6.5 ml of the chloroform phase was transferred into a second clean tube and evaporated to dryness in a water-bath at 60°C under a gentle stream of nitrogen. The dry residue was dissolved in 870 μl of acetonitrile—0.02 *M* potassium dihydrogen phosphate (KH_2PO_4) (5:97, v/v) and 500 μl of this solution were injected into the chromatograph. The extraction scheme is shown in Fig. 1.

Liquid chromatographic conditions

The LDC Constametric IIG high-performance liquid chromatograph was equipped with a Micromeritics 725 automatic injector fitted with a 500-μl injection loop. The chromatograms were recorded with a Perkin-Elmer Model 56 recorder. The stainless-steel column (15 cm × 4.6 mm I.D.) was packed with Spherisorb ODS 5 μm batch 17/49 (Sopares, Gentilly, France), according to the technique described by Broquaire [9]. The flow-rate of the mobile phase (acetonitrile—0.02 *M* KH_2PO_4, 62.5:37.5, v/v) was adjusted to 1.0 ml/min. The detector wavelength was set at 210 nm.

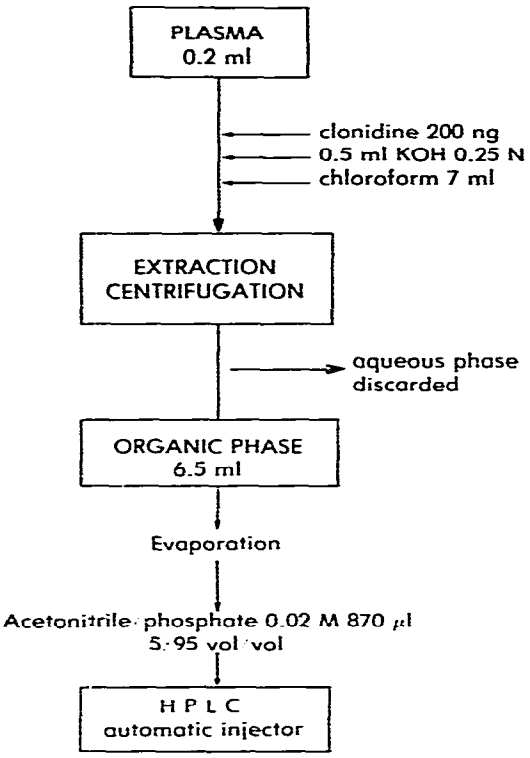


Fig. 1. Scheme for extraction of tolazoline from plasma.

Under these analytical conditions, the retention times were 6.6 and 7.2 min for tolazoline and clonidine, respectively.

RESULTS AND DISCUSSION

First we developed a gas—liquid chromatographic (GLC) method with electron-capture detection. After adding norpethidine (normeperidine) as internal standard, the sample was derivatized with heptafluorobutyric anhydride. This technique was relatively complicated, owing to the difficult acylation of the imidazole nitrogen and the use of norpethidine as internal standard.

Tolazoline absorbs UV light with a maximum at 210 nm, and we therefore decided to develop an HPLC method with clonidine, a structurally related compound, as the internal standard.

The scheme for the extraction of tolazoline from plasma is shown in Fig. 1. The analytical conditions for the extraction of the drug from plasma were established using a spiked human plasma sample containing 5 µg/ml of tolazoline.

A 0.2-ml volume of plasma was acidified with 0.25 *N* hydrochloric acid, made neutral with 0.5 *M* phosphate buffer (pH 7) or alkaline with 0.25 *M* potassium hydroxide solution. Tolazoline was not extracted at acidic pH (ca. 1), poorly extracted at neutral pH and well extracted at alkaline pH (ca. 13).

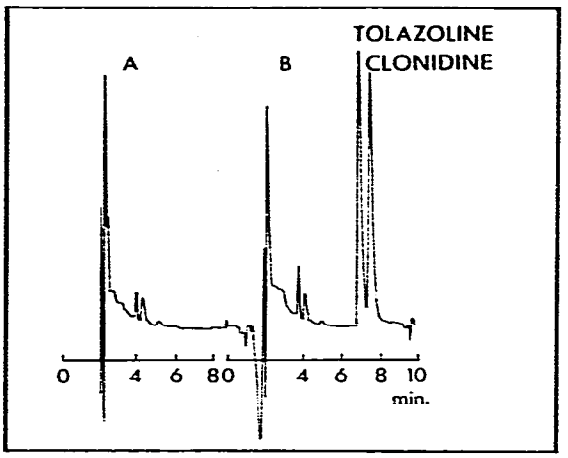


Fig. 2. Chromatograms of plasma extracts. In B, the plasma was spiked with 200 ng of tolazoline and clonidine.

The solvents chloroform, ethyl acetate, freshly distilled diethyl ether and *n*-hexane were tested for optimal extraction. The best solvent was chloroform, which provided after a single extraction at alkaline pH a recovery from the plasma sample of 85 ± 3% (S.E.). Furthermore, the chloroform extract was clean. No endogenous compound present in plasma interfered with tolazoline and clonidine (Fig. 2).

The extraction of tolazoline and clonidine from plasma was completed after 20 min. The UV wavelength selected for the HPLC detector was 210 nm, corresponding to maximum UV absorption for tolazoline. This value gave the best signal-to-noise ratio.

The calibration graph (Table I) was linear for tolazoline plasma concentrations between 0.2 and 20 μg/ml. In these experiments, 200 ng of clonidine were added (20 μl of a 10 μg/ml solution). The minimum sensitivity of detection was 0.2 μg/ml with a coefficient of variation (C.V.) of ± 14.3%. For tolazoline concentrations of 8—20 μg/ml, the coefficient of variation was only 2—3%.

TABLE I

ACCURACY AND REPRODUCIBILITY OF TOLAZOLINE DETERMINATION BY HPLC

Number of determinations: 9.

Added (μg/ml)	Found (μg/ml)	C.V. (%)
0.20	0.21	± 14.3
0.50	0.47	± 8.5
1.00	1.01	± 8.9
4.00	3.96	± 6.3
8.00	8.05	± 2.2
20.00	19.99	± 3.3

Chromatograms obtained from plasma of newborns sampled before and after tolazoline administration are shown in Fig. 2. Gentamicine, cefradine, ampicillin and colistin, drugs that could be associated with tolazoline therapy in newborns, were not co-eluted with tolazoline and the internal standard and do not interfere in the analysis. An example of plasma concentration vs. time curve is displayed in Fig. 3. The newborn received first a loading dose intravenously, followed by an intravenous perfusion to maintain tolazoline plasma levels. At the end of perfusion, the elimination half-life of tolazoline was determined. In this patient, plasma concentrations were stable during perfusion (between 7 and 8 μg/ml), then decreased monoexponentially at the end of perfusion with a half-life of 5.3 h.

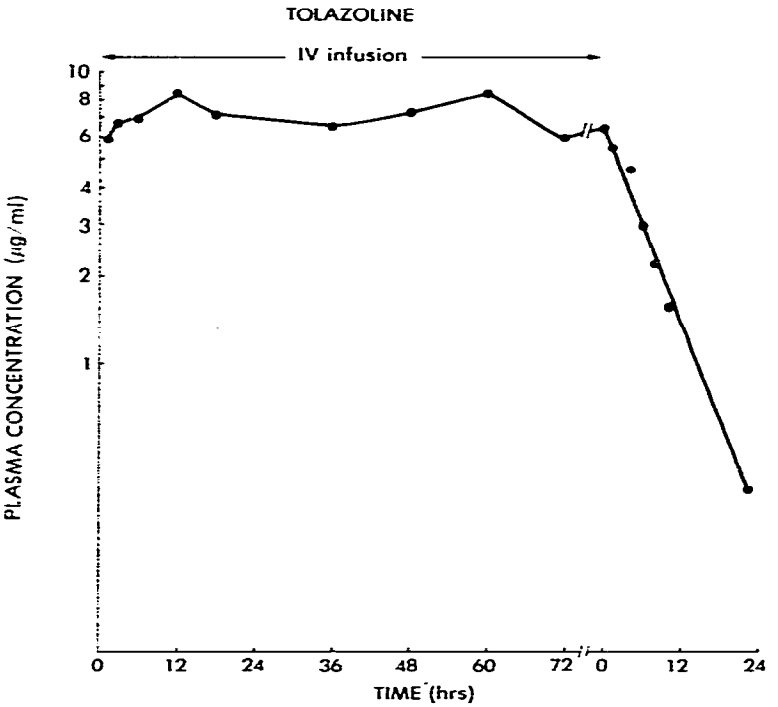


Fig. 3. Plasma concentrations of tolazoline in a 34-week-old newborn (2.38 kg) during and after intravenous infusion. A bolus of 2 mg/kg was injected over a 3-min period, followed by a continuous infusion providing a maintenance dose of 2 mg/kg.

The simplicity of this technique should be emphasized; tolazoline and clonidine can be analysed after a single extraction from alkaline plasma.

The best recovery (85%) was obtained with chloroform, which also provided a clean plasma extract. The reversed-phase conditions allowed the injection of large volumes of the water—acetonitrile mixture, without alteration of the characteristics of the chromatographic separations. Thus, samples could be injected automatically. The chromatographic separation of tolazoline and clonidine was achieved in less than 20 min, and 40—50 samples could be analysed routinely in one day. The described method appears to be suitable for therapeutic plasma monitoring of tolazoline in either newborns or adults.

REFERENCES

1 W.G. Guntheroth, B.C. Morgan, J.A. Harbinson and G.L. Mullins, Circulation, 36 (1967) 724.
2 M. Thomas, H. Campbell and G.E. Heard, Brit. J. Surg., 60 (1973) 545.
3 T.O. Yellin, J.W. Sperow and S.H. Buck, Nature (London), 253 (1975) 561.
4 E. Farkas, Pharmazie, 21 (1966) 600.
5 P.F.G. Boon and W. Sudds, J. Pharm. Pharmacol., 19 (1967) 885.
6 P.P. Quaglio, G. Sandri Cavicchi and G. Cavicchioni, Boll. Chim. Farm., 112 (1973) 760.
7 J.A. Mollica, G.R. Padmanabhan and R. Strusz, Anal. Chem., 45 (1973) 1859.
8 V.J. McLinden and A.M. Stenhouse, Forensic Sci. Int., 13 (1979) 70.
9 M. Broquaire, J. Chromatogr., 170 (1979) 43.