CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Antipyrine and Lorazepam in Human Plasma

Purpose

Determination of antipyrine [F1] and lorazepam [F2] in human plasma.

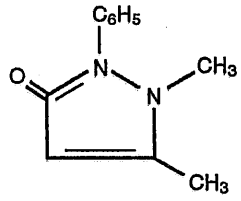

Figure 1. Antipyrine.

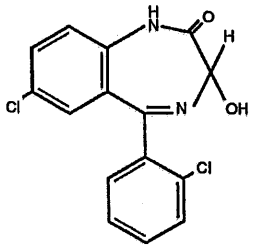

Figure 2. Lorazepam.

Antipyrine is an analgesic commonly used to treat ear infections. Lorazepam is a benzodiazepine tranquilizer. These two drugs are used as models to assess Phase I and Phase II liver function in drug-clearance studies [1]. A new procedure, using salicylanilide as an internal standard, was developed to overcome the limitations of the existing procedure [2].

Existing Method

LCUV, using flunitrazepam as the internal standard. The procedure has several disadvantages: the IS is labile during hydrolysis of the glucuronide conjugate of lorazepam, and there are interfering peaks at the elution times of the IS and lorazepam.

Conditions

System: BAS-200 Liquid Chromatograph.
Detector: UV. See T1 for conditions.
Column: Zorbax Rx, C_8, 250 x 4.6 mm, 7 µm particle size.
Temperature: 30°C.
Mobile Phase: A: 35% (v:v) CH_3CN:65% 7.24 mM sodium phosphate buffer, pH 3.0; B: 80% CH_3CN:20% buffer. See T2 for gradient profile. Flow rate was 1.5 mL/min.
Validation Range: Antipyrine: 5-100 µg/mL
Lorazepam: 10-200 ng/mL.

Sample Preparation

To each 0.25 mL sample aliquot, 0.5 mL of 0.125 M sodium hydroxide was added. The solution was mixed thoroughly and incubated at room temperature for twenty minutes. After the incubation period, 50 µL of 1.25 M sodium phosphate monobasic was added to each sample and then 50 µL of the internal standard working solution (10 µg/mL) was added.

The samples were mixed thoroughly after each addition. Each sample was extracted with 5 mL of methyl *t*-butyl ether. After vortexing and centrifuging, the ether layer was transferred to another tube containing 0.5g sodium sulfate. After vortexing each sample, the ether was decanted into another tube and evaporated under a stream of nitrogen. The dried residue was reconstituted with 500 µL of acetonitrile and 0.1 % (w:v) phosphate buffer (30:70). A 100 µL aliquot was injected onto the column.

Notes

A patient sample is shown in F3 and a blank plasma sample in F4.

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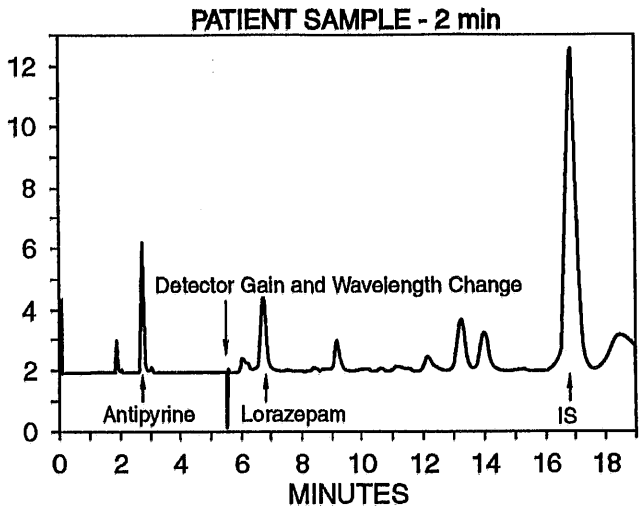

Figure 3. Chromatogram of a sample from a patient.

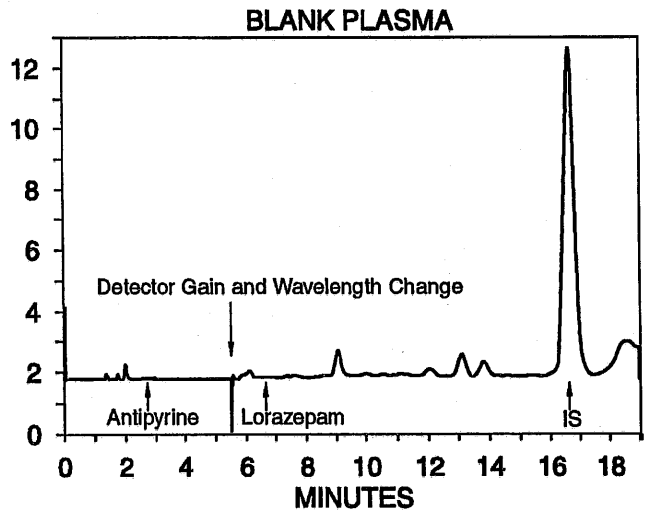

Figure 4. Chromatogram of a plasma blank.

Table 1. Detector schedule.

Time (min)	Wavelength (nm)	Gain (AUFS)	Filter (Hz)
0	254	0.5	1.0
5.5	254	0.5	0.1
5.6	229	0.01	0.1
28.0	254	0.5	1.0

Table 2. Gradient profile.

Time (min)	A (%)	B (%)
0	100	0
15.0	100	0
20.0	20	80
23.0	20	80
28.0	100	0

References

1. *C.A. Riley and W.E. Evans, J. Chromatogr. 382 (1986): 199-205.*
2. *J. Burlage, A.N. Brubaker and L. Grochow, poster presentation at American Association of Pharmaceutical Scientists annual meeting (1991) Washington D.C.*