Journal of Chromatography, 243 (1982) 368–371
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,877

Note

Gas chromatographic determination of Butazolidin (phenylbutazone) in biological fluids

ROBERT D. BUDD
County of Los Angeles, Department of Chief Medical Examiner-Coroner, 1104 No. Mission Rd., Los Angeles, CA 90033 (U.S.A.)
(First received February 3rd, 1982; revised manuscript received March 8th, 1982)

Phenylbutazone (Butazolidin, Sterazolidin) (4-butyl-1,2-phenyl-3,5-pyrazolidinedione) is a very potent drug, used as an analgesic, antipyretic and anti-inflammatory agent. It is orally effective in the treatment of rheumatoid disorders and other non-specific inflammatory disorders. It brings prompt relief of pain, reduces fever and diminishes swelling, tenderness and local heat[1].

Although it is a useful drug, phenylbutazone has serious side-effects. Its use should be carefully monitored by a physician and is not recommended for more than 1 week except in cases of chronic disorders where its use should be very carefully monitored[1].

Because of the dangers of this drug, even when used properly, together with its overdose potential, procedures are needed for monitoring levels of the drug present in blood and urine. Procedures have been developed for the determination of phenylbutazone[2–7], but some of these require plasma or serum[2–5], which is not always obtainable in post mortem cases; others require an electron-capture detector[3], which is generally not available in most laboratories; others are based on UV spectroscopy[6], which is subject to interference by many other drugs, metabolites and decomposition products; still others require lengthy derivatization steps[7]. In this light, a quantitative gas chromatographic procedure has been developed for the determination of phenylbutazone in all biological fluids —whole blood, plasma, urine, bile, etc. (even if decomposed).

Additionally, a fatal overdose case history[8] is presented. The method was used to determine the fatal blood level of phenylbutazone.

EXPERIMENTAL

Reagents

The reagents used were chloroform, spectroanalyzed (Fisher Scientific, Pittsburgh, PA, U.S.A.), concentrated hydrochloric acid, chloroform containing 0.1 μg/ml of Heptabarbital (Geigy, Basel, Switzerland) as extraction solvent, sodium sulphate, anhydrous powder (J. T. Baker, Phillipsburg, NJ, U.S.A.), and 0.5 *N* sodium hydroxide solution. The control solution was 1 μg/ml Butazolidin (Geigy) in water.

0021-9673/82/0000–0000/$02.75 © 1982 Elsevier Scientific Publishing Company

Chromatography

All extraction residues were injected into a Hewlett-Packard gas chromatograph (Model 5700A) equipped with a 6 ft. × 2 mm I.D. glass column packed with 3.8% UCW-982 on 80-100 mesh Chromosorb W HP (Hewlett-Packard, Avondale, PA, U.S.A.).

Procedure

To a 125-ml reagent bottle, add 70 ml of extraction solvent, 1 drop of concentrated hydrochloric acid and 7 ml of the biological fluid (blood, bile, urine, etc.) to be tested or 7 ml of the control solution. Vigorously shake the mixture for 2 min, then filter the mixture through 10 g of sodium sulfate into a 125-ml separatory funnel containing 5 ml of 0.5 *N* sodium hydroxide solution. When the filtration is completed. vigorously shake the contents of the separatory funnel for 2 min. After shaking the contents, allow the layers to separate for about 5 min, then discard the chloroform layer. Transfer 4.5 ml of the sodium hydroxide layer to a clean separatory funnel containing 9 ml of chloroform and 6 drops concentrated hydrochloric acid. Shake the mixture for 1 min, allow it to settle, and then drain the chloroform layer into a 15-ml conical tube. Evaporate the chloroform extract to dryness in a 65°C dry bath under nitrogen. Dissolve the residue in 0.1 ml of chloroform, then inject a 2-μl aliquot of the solution into a gas chromatograph equipped with a 3.8% UCW-982 column run at 240°C. Compare the sample peak retention times with those of the standard control. An internal standard peak must be present in the chromatogram, otherwise the sample was not extracted properly.

Case history

The victim was a 35-year-old white male. He had a medical history of diabetes and epilepsy and had a history of becoming depressed and making suicide threats whenever he became drunk. There was no apparent record of any actual suicide attempts.

The events of the fatal date are unclear. The decedent had been drinking, had taken a large dose of Butazolidin and had ingested something containing lidocaine when he arrived at his sister's home late in the afternoon. He seemed drunk, so his sister refused to let him in. At 5:30 p.m. she observed him unconscious on her front lawn. She tried to arouse him but could not. She called the paramedics, who rushed him to the hospital where he was pronounced dead at 6:42 p.m. Investigation uncovered an empty Butazolidin vial in his pocket.

RESULTS AND DISCUSSION

Using the proposed method, levels of Butazolidin as low as 1 μg/ml can be determined without interferences from other drugs or metabolic products. Basic drugs and neutral drugs are eliminated during the extraction procedure, and other acidic drugs are separated by the UCW-982 gas chromatographic column, as illustrated in Table I. This method easily detects Butazolidin levels within therapeutic ranges, *i.e.*, 50–100 μg/ml[9]. The method is reliable and accurate, as demonstrated by the results of the recovery study presented in Table II.

The proposed method has advantages over other methods[2–7] in that all bio-

TABLE I

SEPARATION OF DRUGS

Substance	*Retention time (min)*
Heptabarbital (internal standard)	4.4
Butazolidin	6.4
Ibomal	2.0
Phenobarbital	2.6
p-Methylphenobarbital	3.1
Alphenal	5.2
Diphenylhydantoin	7.0
p-Methyldiphenylhydantoin	9.0

TABLE II

RECOVERY OF BUTAZOLIDIN

Butazolidin concentration added (μg/ml)	*Mean Butazolidin concentration measured (μg/ml)*	*Standard deviation (μg/ml) (n = 3)*	*Recovery (%)*
20.00	20.30	0.08	101.5
10.00	10.00	0.03	100.0
8.00	7.90	0.03	98.8
2.50	2.13	0.005	85.2

logical fluids can be examined, even decomposed whole blood; an electron-capture detector is not required; lengthy derivatization steps are not required; interferences from other drugs are readily eliminated; an internal standard is included for monitoring the whole extraction procedure and for accurate quantitation.

Heptabarbital is recommended as an internal standard as it is no longer prescribed and should therefore not be present in the biological samples. If it cannot be obtained, ibomal, phenobarbital, *p*-methylphenobarbital, alphenal, or diphenylhydantoin can be used (Table I). On the 3.8% UCW-982 column run at 240°C heptabarbital and Butazolidin have retention times of 4.4 and 6.4 min, respectively, and are therefore well separated.

The decedent studied had the following drug blood levels: alcohol 0.15%, lidocaine 1.5 μg/ml and Butazolidin 109.5 μg/ml. Death was determined to be accidental from acute ethanol and Butazolidin intoxication, complicated by acute pulmonary congestion and edema[8].

The Butazolidin level in the decedent's blood was only slightly higher than the normal therapeutic level, which is achieved from daily dosages ranging from 100 to 600 mg (averaging 400 mg)[1]. While it is known that doses of 5–30 g have proved fatal[9], it is unlikely that the victim ingested more than ten of his 100-mg tablets (1 g of Butazolidin), based on the blood level of Butazolidin found. Thus, Butazolidin overdose could not be the sole cause of death.

The extent and nature of the interaction between alcohol and Butazolidin is

not known. Alcohol is a central nervous system depressant while Butazolidin acts at the site of pain or inflammation, acting on the surface of the mononuclear cells to inhibit their motility[10]. However, death did occur despite the fact that neither Butazolidin nor alcohol was present at overdose levels.

REFERENCES

1 B. B. Huff (Editor), *Physicians' Desk Reference*, Medical Economics Inc., Oradell, NJ, 25th ed., 1970, pp. 722–723.
2 A. Sioufi, F. Caudal and F. Marfil, *J. Pharm. Sci.*, 67 (1978) 243–245.
3 R. B. Bruce, W. R. Maynard and L. K. Dunning, *J. Pharm. Sci.*, 63 (1974) 446–498.
4 R. Perego, E. Martinelli and P. C. Vanoni, *J. Chromatogr.*, 54 (1971) 280–281.
5 I. J. McGilveray, K. K. Midha, R. Brier and L. Wilson, *J. Chromatogr.*, 89 (1974) 17–22.
6 J. J. Burns, R. K. Rose, T. Chenkin, A. Goldman, A. Schulort and B. B. Brodie, *J. Pharmacol. Exp. Ther.*, 109 (1953) 346–357.
7 K. K. Midha, I. J. McGilveray and C. Charette, *J. Pharm. Sci.*, 63 (1974) 1234–1239.
8 *Los Angeles County Coroner's Files*, Case 80-12113, 1980.
9 E. G. C. Clarke, *Isolation and Identification of Drugs*, Pharmaceutical Press, London, 1969, pp. 492–493.
10 M. DiRosa, J. M. Papadimitriou and D. A. Willoughby, *J. Pathol.*, 105 (1971) 239–256.