Determination of Anthranilic Acid in Plasma

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Abstract □ A simple, specific GLC method was developed for the determination of anthranilic acid in plasma. This method is based on extraction from a carefully controlled buffer followed by removal of solvent, silylation, and detection in the gas chromatograph. The procedure is quantitative in the 2–10-μg/ml range.

Keyphrases □ Anthranilic acid—GLC analysis in plasma □ GLC—analysis, anthranilic acid in plasma □ Ascaricides, veterinary—anthranilic acid, GLC analysis in plasma

Anthranilic acid has been used to treat roundworm infestation in animals. Recently, it was identified in the blood of animals receiving the new analgesic 2,3-dihydro-9*H*-isoxazolo[3,2-*b*]quinazolin-9-one[1] (1, 2). Although anthranilic acid also is a naturally occurring body constituent, a sensitive, specific analytical method for its determination in biological fluids has not been described. It has been identified in urine by chromatography (3) and diazotization (4, 5). A nondetailed GC procedure for its detection as the methyl ester has also been cited (6).

This report describes a sensitive, specific quantitative method for the determination of anthranilic acid in plasma.

EXPERIMENTAL

GLC—A dual-column gas chromatograph[2] equipped with a hydrogen flame-ionization detector and a 1-mv recorder[3] was employed. The chromatographic columns used were 2-m × 3-mm i.d. glass tubes packed with 3% XE-60 on 100–120-mesh Gas Chrom Q[4]. The instrument settings were: column temperature, 140°; injection port temperature, 200°; and detector block temperature, 195°. Gas flow rates were: hydrogen, 29 ml/min; and helium (carrier gas), 60 ml/min. Sensitivity settings were: range, 10; and attenuation factor, 8×. The retention times under these conditions were 1.2 min for anthranilic acid and 2.2 min for 4-bromobiphenyl (Fig. 1).

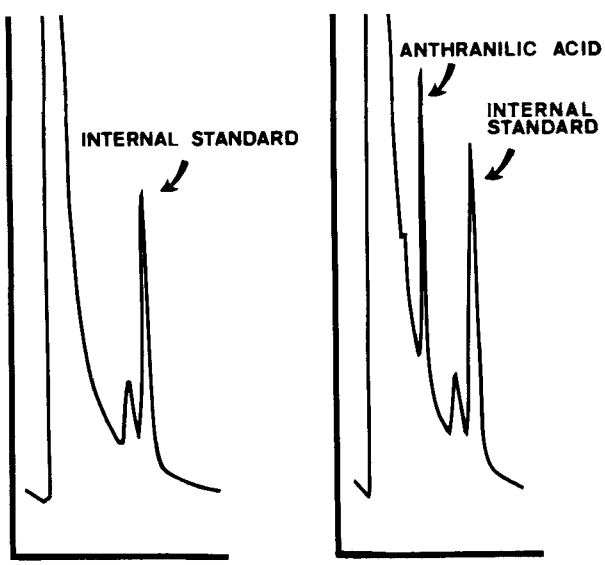

Figure 1—*Gas chromatograms of plasma treated as described. Key: left, chromatogram from normal plasma; and right, chromatogram from normal plasma with anthranilic acid added.*

[1] W2429.
[2] F and M model 402.
[3] Minneapolis-Honeywell.
[4] Applied Science.

Table I—Anthranilic Acid Recovered from Dog Plasma at pH 4.75 by Extraction with Ethyl Acetate

Anthranilic Acid Added to Dog Plasma, μg/ml	Anthranilic Acid Found[a], μg/ml	Anthranilic Acid Extracted[a], %
2.0	2.0	100
5.0	5.0	100
7.0	7.1	102
10.0	10.6	106

[a] Average of duplicates.

Reagents—The reagents were redistilled chloroform, peroxide-free ethyl acetate, 4-bromobiphenyl[5], and bis(trimethylsilyl)acetamide[4].

Procedure—Dog plasma, 1.0 ml, was adjusted to pH 4.75 with 0.3 ml of 2 *M* acetic acid–sodium acetate buffer, pH 4.75. Ethyl acetate, 3 ml, was added, and the mixture was agitated on a mixer for 2 min. After centrifugation for 10 min, 2.0 ml of the organic phase was removed, placed in a 5-ml glass tube, and evaporated to dryness under a nitrogen stream at room temperature.

Chloroform, 0.2 ml, containing 18 μg of 4-bromobiphenyl/ml and 9% bis(trimethylsilyl)acetamide was added to the residue, and it was stirred on a mixer for a few seconds. A 2-μl aliquot of this solution was injected into the gas chromatograph. The concentration of anthranilic acid was determined by the relative peak height method, using 4-bromobiphenyl as the internal standard.

RESULTS AND DISCUSSION

The relative peak heights (±*SE*) for 2, 5, 7, and 10 μg of anthranilic acid/ml were 0.37 ± 0.0053, 0.96 ± 0.0091, 1.34 ± 0.0008, and 2.00 ± 0.0102, respectively. The reproducibility of the procedure is indicated by the standard error of quadruplicate determinations.

The extraction technique effectively separates anthranilic acid from normally interfering plasma constituents since determinations with normal plasma give little or no blank (Fig. 1). The pH of the ethyl acetate

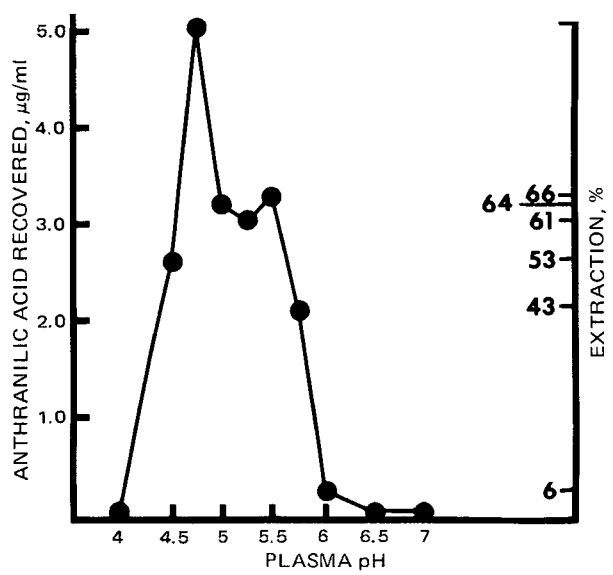

Figure 2—*Effect of pH on the extraction of 5.0 μg/ml of anthranilic acid from plasma.*

[5] Eastman Chemical Co.

extraction is critical. Recovery studies at pH 4.75 showed complete extraction of anthranilic acid over the effective assay range of 2–10 μg/ml (Table I); but at a slightly higher pH level of 5.0 or at a slightly lower level of 4.5, only about one-half of the anthranilic acid was extracted (Fig. 2).

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Detection of Penicillin G and Ampicillin as Contaminants in Tetracyclines and Penicillamine

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Abstract □ A method was developed to detect residual levels of ampicillin and penicillin G in various tetracyclines and penicillamine. Residues are detected by reversed-phase TLC followed by bioautography. The directness of the techniques makes this method a good means of detecting residual contaminants in drugs.

Keyphrases □ Penicillin G—TLC–bioautographic analysis as contaminant in various tetracyclines and penicillamine □ Ampicillin—TLC–bioautographic analysis as contaminant in various tetracyclines and penicillamine □ TLC–bioautography—analysis, penicillin G and ampicillin as contaminants in various tetracyclines and penicillamine □ Tetracyclines, various—TLC–bioautographic analysis of penicillin G and ampicillin as contaminants □ Penicillamine—TLC–bioautographic analysis of penicillin G and ampicillin as contaminants □ Antibacterials—TLC–bioautographic analysis of penicillin G and ampicillin as contaminants in various tetracyclines and penicillamine

The problem of penicillin contamination in nonpenicillin products was recognized as early as 1964. An ad hoc Advisory Committee on Penicillin Contamination composed of experts in the fields of allergy and penicillin therapy (1) was convened by the Commissioner of Food and Drugs to evaluate the potential public health problems. The potential danger of allergic reactions, ranging from minor symptoms to fatal anaphylaxis, and the possibility of sensitizing individuals by repeated trace doses were considered.

Based on the data then available, limits on the amount of penicillin allowable in nonpenicillin drugs were recommended. These limits were less than 0.05 unit/maximum single dose for parenteral drugs and less than 0.5 unit/maximum single dose for oral drugs. Methods to detect penicillin G were specified by the Food and Drug Administration (2).

BACKGROUND

In 1974, a screening study (3) was conducted to ascertain which, if any, of the other penicillins could be detected in erythromycin and tetracycline by existing methods. That study showed that the current methods were less sensitive to residual levels of phenethicillin, methicillin, nafcillin, oxacillin, and cephalothin and were completely incapable of detecting residual ampicillin.

This 1974 evaluation indicated the need for new methods to detect residual ampicillin. Investigations conducted in 1974 resulted in a new bioautographic method (4) to separate and detect as little as 1 ppm of ampicillin from tetracycline hydrochloride bulk powder.

In 1975, new procedures (5) were described for the detection of ampicillin and penicillin G contaminants in demeclocycline and chlortetracycline. Although analytical capabilities had been expanded, additional methods were needed to detect ampicillin and penicillin G in doxycycline, oxytetracycline, and methacycline. Biagi *et al.* (6) described a reversed-phase TLC method to determine partition data for the penicillins. Using various concentrations of acetone in the mobile phase (6) of this system, they varied the R_f values of 11 penicillins and concluded: "The most hydrophilic compounds are the first to reach a maximum R_f value. On the other hand, at 0% acetone in the mobile phase the most lipophilic compounds remained close to the origin" (6).

The possibility that this type of system might be applied to residual contamination of other antibiotics seemed feasible. The techniques described by Biagi *et al.* (6) were applied to the detection of residual ampicillin and penicillin G in the tetracyclines and residual penicillin G in penicillamine.

EXPERIMENTAL

Preparation of TLC Plates—TLC plates[1], 20 × 20 cm, precoated with silica gel GF[2], were impregnated with silicone[3] by developing the plates in 200 ml of silicone–ether[4] (5:95 v/v) in a covered developing chamber. After overnight development, the plates were removed from the chamber and allowed to air dry at room temperature.

Sample Preparation—With glass-stoppered volumetric flasks to prevent evaporation of the solvent, standard solutions of ampicillin were prepared in acetone[5] to contain 0.1, 0.05, 0.025, 0.0125, and 0.00625 μg/ml. A similar set of penicillin G standards was prepared. Portions of 25 mg of chlortetracycline (I), demeclocycline (II), methacycline (III), minocycline (IV), oxytetracycline (V), tetracycline (VI), and doxycycline hyclate (VII) were placed in small (7 ml) glass-stoppered weighing vials. One milliliter of each standard solution was added to the vials containing the tetracyclines; then the vials were stoppered and gently shaken for a few seconds to form a suspension of the drugs.

This same procedure was followed for a second set of samples using the penicillin G standards. In addition, 250-mg portions of penicillamine

[1] Analtech, Inc., Blue Hen Industrial Park, Newark, DE 19711.

[2] Brinkmann Instruments, Westbury, NY 11590.

[3] Silicone DC 200 (350 centistokes), Applied Science Laboratories, State College, PA 16801.

[4] Burdick & Jackson Laboratories, Muskegon, MI 49442.

[5] Fisher Scientific, Fair Lawn, N.J.