# Thin-Layer Chromatographic Identification of Organoarsenical Feed Additives in Feeds

A thin-layer chromatographic procedure for the identification of organoarsenical feed additives in feeds is described. This method utilizes an extraction with methanol and subsequent thin-layer chromatography on silica gel G. Although roxarsone and arsanilic acid migrate with the same $R_f$, they can be readily distinguished from each other by spraying the developed plate with dimethylaminocinnamaldehyde or dimethylaminocinnamaldehyde-$TiCl_3$, reagents specific for the aromatic amino and aromatic nitro group, respectively. With the solvent systems used, carbarsone cannot be satisfactorily separated from arsanilic acid. The detection limit for these arsenicals is $\geqq 0.25$ $\mu$g.

Only five organoarsenicals are currently approved for use in medicated feeds. These drugs are roxarsone (4-hydroxy-3-nitrobenzenearsonic acid), nitarsone (4-nitrobenzenearsonic acid), arsanilic acid (4-aminobenzenearsonic acid), carbarsone (*p*-ureidobenzenearsonic acid), and arsenosobenzene. They are used to medicate more than 12 million tons of feed annually in the United States.

Even though assay procedures for these drugs in feeds exist (Association of Official Agricultural Chemists, 1965a,b; Cavett, 1956a,b), and $R_f$ values for pure forms of these drugs obtained by paper chromatography (Mitchell, 1959) and thin-layer chromatography (Antkowiak and Spatorico, 1967) have been reported, there are no reports available describing a convenient technique for the identification of an unknown arsenical feed additive when present in feeds.

The purpose of this work was to develop a rapid technique which could be used by feed laboratories or regulatory agencies for the identification of any of the more commonly used arsenical feed additives in feeds either alone or in combination with other approved drugs. Arsenosobenzene, an arsenical of limited use, is not detected by this technique.

## EXPERIMENTAL

**Reagents.** DMC spray reagent (for arsanilic acid and carbarsone). Dimethylaminocinnamaldehyde (DMC) 0.1%, in 50% acetic acid. Dissolve DMC in glacial acetic acid and add an equal volume of water.

DMC-$TiCl_3$ spray reagent (for roxarsone and nitarsone). Add 1 ml. of 20% titanium trichloride to 25 ml. of the DMC reagent. Prepare fresh and use within 30 minutes after mixing.

Roxarsone and nitarsone, purified (Salsbury Laboratories, Charles City, Iowa).

Carbarsone (Whitmoyer Laboratories, Inc., Myerstown, Pa.).

Reference standards, 0.2 mg. per ml., in methanol.

**Extraction.** Extract 20 grams of feed with 75 ml. of methanol. Filter. Add 10 ml. of concentrated ammonium hydroxide and 5 grams of charcoal (Darco G-60) to the filtrate, swirl, and let stand 5 to 10 minutes. Filter and concentrate the filtrate to a small volume (less than 5 ml.) on a steam bath under a stream of air.

**Thin-Layer Chromatography.** Prepare 0.25-mm. thick silica gel G (Brinkman Instrument Co.) thin-layer plates and air-dry overnight. Activation of the layer is not recommended since heating at 100° C. destroys the ability of the silica gel to separate roxarsone from nitarsone. Spot 5 to 10 $\mu$l. of the concentrated extract and 5 to 10 $\mu$l. of the reference standards on the plate and develop in acetonitrile-water-ammonia (65:30:5) or ethanol–ammonium hydroxide (1 to 1) to a height of 8 to 10 cm. Equilibration of the chromatographic chamber with solvent vapors is not necessary. To differentiate between roxarsone and arsanilic acid, spray the developed plate with the DMC spray reagent to detect the presence of arsanilic acid. Roxarsone can be detected by overspraying with the DMC-$TiCl_3$ spray reagent. In these solvent systems, carbarsone was not satisfactorily separated from arsanilic acid.

## RESULTS AND DISCUSSION

The level or concentration range of the medicament in the feed is given in Table I. An extraction and cleanup system suitable for any of these four arsenical feed additives in feed was essential. Extraction with methanol

**Table I. Approved Use Levels of Organoarsenical Feed Additives**

| Arsenical | Use Level in the Feeds | |
|---|---|---|
| | % | P.p.m. |
| Arsanilic acid | 0.005–0.01 | 50–100 |
| Arsenosobenzene | 0.002 | 20 |
| Carbarsone | 0.0375–0.1 | 375–1000 |
| Roxarsone | 0.0025–0.005 | 25–50 |
| Nitarsone | 0.01875 | 187 |

and treatment of the extract with charcoal satisfied this requirement.

The acetonitrile–water–ammonium hydroxide (65:30:5) chromatographic solvent system used by Mitchell (1959) in his two-dimensional paper chromatographic procedure for arsenicals was applicable to silica gel thin-layer chromatography as well. However, his second solvent system, acetonitrile–water–nitric acid (78:20:2), was unsatisfactory since the four arsenical feed additives migrated with the solvent front. An equally efficient solvent system, ethanol–ammonium hydroxide (1 to 1), has also been used in this laboratory (Table II).

The TLC solvent systems satisfactorily separated the extracted arsenical feed additives from the following drugs with which they are often combined: acetyl-(*p*-nitrophenyl)-sulfanilamide (sulfanitran); 3,5-dinitrobenzamide (nitromide); 2-chloro-4-nitrobenzamide (aklomide); 3,5-dinitro-*o*-toluamide (zoalene); dibutyltin dilaurate; *N*,*N*'-di-(3-nitrobenzenesulfonyl)-ethylene diamine (dinsed); 1-(4-amino-2-*n*-propyl-5-pyrimidinylmethyl)-2-picolinium chloride hydrochloride (amprolium); and methyl-4-acetamido-2-ethoxybenzoate (ethopabate).

The DMC spray reagent, which produces red to blue spots with aromatic amines, was satisfactory for the detection of these arsenicals. The addition of $TiCl_3$ to the DMC reagent enables the spray reagent to reduce aromatic nitro groups to the corresponding amine. The resulting amine then couples with the DMC reagent to produce the red-to-blue colors. Because of the residues of ammonia from the TLC solvent, the spray reagents produce a yellow-orange background color on the plate which increases in intensity with time. The colored spots also tend to fade after several hours. Therefore, to obtain valid results, the plates must be read within 15 minutes after spraying.

An alternate spray reagent is 0.1% *p*-dimethylaminobenzaldehyde (PDAB) which produces yellow or orange colors under the same conditions used for the DMC reagent (Table II). However, this reagent is subject to the same disadvantages of the DMC reagent.

The limit of detection of these arsenicals in pure form is ≧ 0.25 μg. with either the DMC or DMC-$TiCl_3$ spray.

**Table II. TLC $R_f$ Values of Organoarsenical Feed Additives on Silica Gel G**

| | Solvent[a] | | Detection[b] | | | |
|---|---|---|---|---|---|---|
| | | | | DMC- | | PDAB- |
| Arsenical | 1 | 2 | DMC | $TiCl_3$ | PDAB | $TiCl_3$ |
| Arsanilic acid | 0.30 | 0.35 | Red | Red | Yellow | Yellow |
| 4-Hydroxy-3-nitrobenzenearsonic acid | 0.33 | 0.44 | — | Red | — | Yellow |
| 4-Nitrobenzene arsonic acid | 0.55 | 0.63 | — | Red | — | Yellow |
| *p*-Ureidobenzenearsonic acid | 0.30 | 0.35 | Red | Red | Yellow | Yellow |

[a] 1 = acetonitrile–water–ammonium hydroxide (65:30:5). 2 = ethanol–ammonium hydroxide (1 to 1).
[b] DMC = *p*-dimethylaminocinnamaldehyde. PDAB = *p*-dimethylaminobenzaldehyde.

Therefore, under the conditions of the described extraction and TLC procedure, it would be possible to detect less than 10 p.p.m. of any of these arsenicals in the feed—levels which are considerably less than the lowest approved level for these drugs.

LITERATURE CITED


Antkowiak, J. J., Spatorico, A. L., *J. Chromatog.* **29,** 277 (1967).
Association of Official Agricultural Chemists, "Official Methods of Analysis," 10th ed., p. 632, 33.001, Washington, D. C., 1965a.
Association of Official Agricultural Chemists, "Official Methods of Analysis," 10th ed., p. 637, 33.031, Washington, D. C., 1965b.
Cavett, J. W., *J. Assoc. Offic. Agr. Chemists* **39,** 857 (1956a).
Cavett, J. W., *J. Assoc. Offic. Agr. Chemists* **39,** 967 (1956b).
Mitchell, L. C., *J. Assoc. Offic. Agr. Chemists* **42,** 161 (1959).



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