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Note

Thin-layer chromatography of various analgesics, antipyretics and anti-inflammatory agents

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A number of thin-layer chromatographic systems has previously been described for the separation of compounds of pharmaceutical interest having analgesic, antipyretic or anti-inflammatory properties.[1] More recently, separation techniques have been reported for these types of compounds employing polyamide[2], polyamide–Kieselguhr[3] and silica gel layers[4–12]. Such studies were primarily restricted to a limited class of compounds, such as the salicylic acid or pyrazolone derivatives, or consisted of only a few representative compounds within each class.

In this communication, we report a thin-layer chromatographic procedure utilizing pre-coated silica gel sheets that has been applied to the characterization of twenty-nine different compounds including derivatives of salicylic acid, aniline, pyrazolone and 3,5-pyrazolidinedione as well as a number of miscellaneous compounds that exhibit these pharmacological effects.

EXPERIMENTAL

Materials

All solvents were analytical reagent grade and used without further purification. Mefenamic acid was obtained from Parke, Davis & Co. (Detroit, Mich., U.S.A.) and indomethacin from Merck (Rahway, N.J., U.S.A.). Pentazocine free base was supplied by Winthrop Labs. (Rensselaer, N.Y., U.S.A.). The remaining reference compounds were USP or NF grade or obtained through commercial sources. Solutions of each of the reference compounds were prepared just prior to use at a concentration of 5 μg/μl in ethanol–chloroform (1:1). When necessary, a minimum amount of water was added dropwise to dissolve the compounds. Eastman Chromagram polyester sheets 6060 (20 × 20 cm) coated with silica gel (100 μm) and incorporated with a fluorescent indicator were used. These sheets were stored in a desiccator, non-activated, and prior to use, 7 mm of adsorbent were removed by scraping from the bottom, left and right edges.

Thin-layer chromatography

The silica gel sheets were spotted by means of capillary micropipettes (3–5 μl) at a distance of 1 cm from the bottom edge of the adsorbent layer at 1-cm intervals and

then placed in equilibrated tanks (9 × 23 × 23 cm) lined with Whatman No. 3MM chromatographic paper saturated with freshly prepared mobile phase approximately 1 h prior to development. The chromatograms were developed by the one-dimensional ascending technique to a height of 15 cm (50 min) under ambient conditions employing cyclohexane–acetone–acetic acid (40 : 50 : 1) as the mobile phase. After development and air drying at room temperature, visualization of the spots was carried out by UV irradiation at 254 nm which revealed distinct blue or purple spots against a pink fluorescent background. Mefenamic acid was observed as a brown spot under these conditions.

TABLE I

CHROMATOGRAPHIC RETENTION DATA ON SILICA GEL

Mobile phase: cyclohexane–acetone–acetic acid (40:50:1). Values for secondary spots are shown in parentheses.

Compounds	R_F	*Relative* R_F*
Salicylic acid and derivatives		
Salicylic acid	0.41	0.73
Sodium salicylate	0.41	0.73
Strontium salicylate	0.41	0.73
Acetylsalicylic acid (Aspirin)	0.54	0.96
o-Salicylsalicylic acid	0.62	1.11
p-Aminosalicylic acid**	0.43	0.77
Methyl salicylate	0.69	1.23
Phenyl salicylate	0.70	1.25
Salicylamide	0.60	1.07
Aniline derivatives		
Acetanilide	0.58	1.04
Acetaminophen	0.42	0.75
Phenacetin	0.56	1.00
Pyrazolone derivatives		
Antipyrine	0.34	0.61
Aminopyrine	0.50	0.89
Dipyrone	0.00	0.00
3,5-Pyrazolidinedione derivatives		
Phenylbutazone	0.66 (0.61)	1.18 (1.09)
Oxyphenbutazone	0.58 (0.53)	1.04 (0.95)
Sulfinpyrazone	0.16	0.29
Miscellaneous compounds		
p-Aminobenzoic acid***	0.48	0.86
Indomethacin	0.56	1.00
Mefenamic acid	0.63	1.12
Propoxyphene·HCl	0.08	0.14
Pentazocine	0.13	0.23
Phenyramidol·HCl	0.44	0.79
Quinine·HCl	0.07	0.12
Procaine·HCl	0.03	0.05
Prednisone	0.46	0.82
Cortisone acetate	0.55	0.98
Hydrocortisone	0.46	0.82

* Relative to phenacetin.
** Compound employed as a tuberculostatic.
*** Compound employed to potentiate salicylate activity.

RESULTS AND DISCUSSION

The retention data for the twenty-nine compounds shown in Table I represent the mean of four determinations carried out on four different days. The relative R_F values, calculated with respect to the migration of phenacetin, were determined for each of the compounds by including this particular reference standard on all of the sheets. The additional spot observed for both phenylbutazone and oxyphenbutazone is possibly due to the 4-hydroxy derivative or to a product from the hydrolytic or oxidative cleavage of the pyrazolidine ring system. The presence of such conversion products from the decomposition of phenylbutazone and its sodium salt has previously been demonstrated and the resulting compounds identified[13,14]. In addition to exhibiting a spot under UV irradiation, mefenamic acid was also visible directly as a bright yellow spot.

In general, the detection limits for the method are estimated at the 1–5-μg level. The procedure as described has been used effectively in routine qualitative applications with pharmaceutical preparations containing a number of the listed compounds.

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