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Note

Detection of some non-steroidal anti-inflammatory agents on thin-layer chromatographic plates coated with fluorescent mixtures

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Compounds separated by thin-layer chromatography (TLC) that absorb in the ultraviolet region but without emission in the visible region can be detected using thin layers impregnated with luminophores. Such layers show ultraviolet fluorescence, the colour being determined by the luminophore used, and the separated compounds appear as dark spots, usually at 254 nm. The most popular plates with luminophores are those pre-coated with silica gel F_{254} (Merck) containing zinc orthosilicate (green fluorescence at 254 nm).

The sensitivity of this method depends on the molar absorptivities and the coincidence between the excitation spectrum of the luminophore and the absorption spectrum of the sample substances.

Although the luminophores used in TLC are very efficient in detecting separated compounds, the selectivity they offer is poor because the substances appear as colourless spots. Tamura[1] suggested the use of a luminophore mixture in order to enhance the detection selectivity because there is the possibility of revealing the compounds as coloured spots on such layers Tamura *et al.*[2] discussed diverse luminophore mixtures with nearly white fluorescence at 254 nm, which made it possible to detect some organic compounds as coloured spots.

Nakamura and Tamura[3,4] used mixed fluorescent thin layers of strontium pyrophosphate activated by tin (blue fluorescence), zinc orthosilicate activated by manganese (green fluorescence) and yttrium vanadate activated by europium (red fluorescence) in the ratio 20:5:1 for detection of some sulphur-containing compounds as coloured spots at 254 nm. They also used the mixed fluorescence technique for the qualitative detection of some inorganic ions[5].

Okumura[6] used partially crystallized fluorescent glass to prepare sintered plates with a luminophore mixture in order to separate and detect water-soluble vitamins and some inorganic ions.

We prepared plates with silica gel R and a mixture of magnesium fluorogermanate activated by manganese (red fluorescence), zinc orthosilicate activated by manganese (green fluorescence) and zinc sulphide activated by silver (blue fluorescence) in the ratio 6:9:2 in order to separate and detect some purines[7] and benzaldehydes[8] as coloured spots at 254 nm on a nearly white fluorescent background.

In this paper we compare the results for the detection of some non-steroidal anti-inflammatory agents on plates with a luminophore mixture of magnesium fluo-

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rogermanate, zinc orthosilicate and zinc sulphide and on plates with a mixture of magnesium fluorogermanate activated by manganese (red fluorescence), zinc–magnesium germanate activated by manganese (green fluorescence) and calcium tungstate (blue fluorescence) at 254 nm on a nearly white background.

EXPERIMENTAL

The non-steroidal anti-inflammatory agents ibuprofen, oxyphenbutazone, piroxicam, diclofenac, indomethacin, fenbufene, aspirin and phenylbutazone were supplied by Terapia (Cluj-Napoca, Rumania).

The following solutions of concentration 2 mg/ml were prepared: ibuprofen and oxyphenbutazone in ethanol, piroxicam, diclofenac, indomethacin and phenylbutazone in ethanol–acetone (1:1), fenbufene in benzene and aspirin in water. From these or more dilute solutions, 1 or 2 μl were applied on plates using Brand micropipettes.

Thin layers 0.5 mm thick on glass plates (20 × 20 cm) were prepared using silica gel R (Institute of Chemistry, Cluj-Napoca, Rumania) with 1% starch–agar-agar (1:1) as binder to which the mixtures of magnesium fluorogermanate, zinc orthosilicate and zinc sulphide (7:8:2) was added.

A hot water suspension was homogenized in a ball-mill for 45 min, then it was spread on glass plates. The plates were left to dry in air until the next day. Plates of magnesium fluorogermanate, zinc orthosilicate and calcium tungstate (7:8:4) were prepared in a similar manner. All luminophores are commercially available from the Institute of Chemistry, Cluj-Napoca, Rumania.

Chromatography was performed in a normal developing chamber with chloroform–methanol–25% ammonia (18:15:5) after saturating the chamber for 20 min. All solvents were obtained from Reactivul (Bucharest, Rumania) and were used as received. After being developed for a distance of 10 cm for 45 min, the plates were dried first in an air current and then in a drying chamber at 100–120°C for 15 min and were observed under ultraviolet light at 254 nm with a Camag lamp.

RESULTS AND DISCUSSION

The results obtained on the two types of plates in comparison with plates pre-coated with silica gel F_{254} (Merck) are shown in Table I.

Although it is difficult to differentiate between the two types of plates with the luminophore mixture taking into account the colours of these compounds, the plates with magnesium fluorogermanate, zinc orthosilicate and zinc sulphide show superior sensitivity. All compounds appear after separation on these plates with a visual detection limit of 1 μg per spot at 254 nm. Detection on the Merck plates is difficult at this concentration level and is not possible for all of the compounds studied.

This paper emphasizes the results reported in previous papers, namely that the mixed luminophore detection method, which is simple and non-destructive, affords more advantages than single luminophore plates with respect to selectivity and sensitivity.

TABLE I

SEPARATION AND DETECTION OF SOME NON-STEREOIDAL ANTI-INFLAMMATORY AGENTS ON SILICA GEL PLATES WITH SINGLE AND MIXED LUMINOPHORES

Compound	hR_F*	*Colour at 254 nm*		
		*A**	*B***	*C****
Ibuprofen	27	Violet	–	–
Oxyphenbutazone	12	Violet	Red-brown	Brown
Piroxicam	34	Green	Bluish	Brown
Diclofenac	44	Grey	Mauve	Brown
Indomethacin	49	Brown	Brown	Brown
Fenbufene	37	Blue	Bluish	Brown
Aspirin	16	Bluish	Violet	Blue
Phenylbutazone	62	Violet	–	–

* Plate with magnesium fluorogermanate, zinc orthosilicate and zinc sulphide.
** Plate with magnesium fluorogermanate, zinc–magnesium germanate and calcium tungstate.
*** Pre-coated silica gel F_{254} plate (Merck).

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