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A New Reagent for Detection of Barbiturates by Thin-layer Chromatography

Barbitüratların İnce Tabaka Kromatografisiyle Teşhisi için yeni bir Reaktif

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The analyses of the barbiturates has received often repetitive attention from many authors. Most of the work was designated for the identification of barbiturates in blood, urine, tissue samples or in multicomponent tablets by paper and thin-layer chromatography[1-7]. Whatman No. 1 filter paper or Silica Gel G were the most common adsorbant. Different solvents were investigated and chloroform-acetone, chloroform-benzene-ammonia, chloroform-methanol, isopropanol-chloroform-ammonia were the most popular systems used.

Many detection reagents such as mercurous salts[6,10], mercuric salts with diphenylcarbazone[8,9], cobalt nitrate[11], silver acetate[7], silver acetate-diphenylcarbazone[5], mercury-dithizone[12] were used.

The color of the spots obtained with mercury or silver containing reagents are not stable. This ist the most important point of the problem.

We would like to report a simple reagent for identification of six barbiturates.

Ninhydrin, sodium 1,2-naphtoquinone sulfonate, rosaniline, methyl violet, bromcresol green, diphenylamine, thymolphtalein, fluorescein and rhodamine B were tested as spray reagent with or without mercurous nitrate. Rhodamine B with mercurous nitrate was found to be the best spray reagent for detecting the examined barbiturates which give a purplish pink spots on a light pink background. This color is stable for a longtime and the sensitivity with this reagent is very high.

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EXPERIMENTAL

Tablets * containing barbital, phenobarbital, pentobarbital, itobarbital, cyclobarbital and powder ** containing thiopental were investigated by thin-layer chromatography.

Extraction. As a routine procedure the components were dissolved in water, after filtration the barbiturates were liberated from their salts by the addition of hydrochloric acid and extracted with chloroform.

Thin-layer plates. 20×20 cm plates coated with Silica Gel G were used and activated at 120° for 20 min.

Solvents. Chloroform-acetone-ammonia 25% (10:18:2) (S_1) or (10:14:2) (S_2).

Reagents. I — a) 0.1% solution of rhodamine B in EtOH mixed with equal part of volumes of 1% solution of mercurous nitrate. b) 1% solution of mercurous nitrate in water, acidified with nitric acid. The chromatogram sprayed first with (a) and then with (b). The barbiturates appear as purplish pink spots on a light pink background. The Rf values and minimum detectable quantities are shown in table I.

Table I. Rf values and sensitivities

Drug	$Rf(S_1)$	$Rf(S_2)$	sensitivitiy, μg
Barbital	0.27	0.37	5
Phenobarbital	0.20	0.31	1.5
Pentobarbital	0.50	0.56	3
Itobarbital	0.41	0.46	1.5
Cyclobarbital	0.37	0.42	3
Thiopental	0.69	0.60	3

II — Ninhydrin. To a 0.3% solution of ninhydrin in n-butanol, 3 ml of glacial acetic acid was added.

III — Sodium 1,2-naphtoquinone sulfonate, 0.1% in EtOH.

IV — Bromcresol green, 0.1% in EtOH, followed by mercurous nitrate, 1%.

V — Diphenylamine, 0.1% in MetOH.

* Medinal (barbital sodium), Plexonal (barbital sodium, itobarbital sodium and phenobarbital sodium), Muhadorm and Nembutal (pentobarbital sodium), Ipnos (cyclobarbital calcium).

** Pentothal (thiopental sodium).

Table II. Color obtained with other reagents

Drug	Reagents															
	II After irradiation with UV (366 mμ)		III		IV		V		IV After irradiation with UV (254 mμ)		VII		VIII		IX	
	c.s	b	c.s	b	c.s	b	c.s	b	c.s	b	c.s	b	c.s	b	c.s	b
Barbital	violet	pale purple	0	0	0	0	White	light green	0	0	0	0	White	dark red	0	0
Phenobarbital	»	»	0	0	violet	yellow	»	»	0	0	0	0	*		0	0
Pentobarbital	»	»	violet	pink	0	0	»	»	violet	green	0	0	White	dark red	yellow	green
Itobarbital	0	0	»	»	0	0	»	»	0	0	0	0	»	»	»	»
Cyclobarbital	violet	pale purple	0	0	0	0	»	»	violet	green	violet	green	»	»	»	»
Thiopental	»	»	violet	pink	0	0	»	»	»	»	»	»	»	»	»	»

c. s: color of the spots, b: background, o: not detectable

*. violet spot appears, after spraying with mercurous nitrate as second reagent the spot remains violet but the background becoms purple.

VI — Thymolphtalein, 0.1 % in MetOH.
VII — Methyl violet, 0.05 % in EtOH, followed by mercurous nitrate 1 %.
VIII — Rosaniline, 0.1 % in EtOH.
IX — Fluorescein, 1 % in EtOH, followed by mercurous nitrate 1 %.

Confirmation was obtained by spraying the chromatograms with appropriate reagent. Some reagent gives color after irradiation with UV light (254 or 366 mμ). The color obtained are illustrated in Table II.

SUMMARY

Different reagents were investigated for detection of barbital, phenobarbital, pentobarbital, itobarbital, cyclobarbital and thiopental on thin-layer chromatograms. Rhodamine B with mercurous nitrat found to be the best one giving a stable purplish pink color. The sensitivity with this reagent is very high, especially for phenobarbital and itobarbital.

ÖZET

Barbiturat ihtiva eden tabletlerden hidroklorik asid ile serbest hale getirilen ve kloroformla ekstre edilen barbital, fenobarbital, pentobarbital, itobarbital, siklobarbital ve ayrıca tiopentalin ince tabaka kromatografisile teşhisleri yapıldı.

Adsorban, 120° de 20 dakika müddetle aktive edilmiş Silika Gel G, eriticiler ise kloroform-aseton-amonyak % 25 (10:18:2) veya (10:14:2) sistemleridir.

Kromatogramlara muhtelif reaktifler tatbik edilmiş, birbirini takiben püskürtülen Rhodamine B-merküro nitrat ve merküro nitrat reaktifi ile açık pembe zemin üzerinde uzun zaman sabit kalan erguvani pembe lekeler elde edilmiştir. Bu reaktif ile sansitivite büyük olup fenobarbital ve itobarbital için sansitivite 1.5 μg olarak tespit edilmiştir.

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