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Analysis of Pharmaceuticals Containing Antihistamines by Quantitative Thin-layer Chromatography

Antihistaminik Madde İhtiva Eden İlâçların Kantitatif İnce Tabaka Kromatografisi

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The wide-spread use of antihistaminic preparations has resulted, in the development of analytical techniques for the identification and assay of these compounds.

Macek and Vacerkova(1) used paper and thin-layer chromatography, Gendi et al.(2), Fike and Sunshine(3) used thin-layer chromatographic technique for the identification of antihistamines.

As the usual color reactions for detection of the antihistamine spots are based on the reactions of amine group or intracyclic nitrogene, Dragendorff reagent(4), p-dimethylaminobenzaldehyde(4), ceric sulfate(5), Pauly reagent, potassium iodoplatinate, ammonium vanadate-sulfuric acid were used for this purpose.

Different methods were proposed for the quantitative determination of antihistamines. The United States Pharmacopeia(6) and the British Pharmacopoeia(7) utilize nonaqueous titrimetric or ultraviolet spectrophotometric methods. Blaug and Zopf(8) proposed an ion exchange separation for the determination of diphenhydramine HCl, doxylamine HCl, chlorpheniramine maleate and tripellenamine HCl, alone or in pharmaceutical preparations. Morrison and Chatten(5) proposed a quantitative thin-layer technique which is based on the careful measurement of spot area from which the concentration of the substance can be calculated.

This paper deals with the identification by TLC and estimation by quantitative thin-layer technique of the below mentioned antihistamines in solution or in multicomponent pharmaceuticals.

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EXPERIMENTAL

Pheniramine (I), chlorpheniramine (II), brompheniramine (III), and pyrilamine (IV) maleates, thonzylamine HCl (V), diphenhydramine HCl (VI), phenyltoloxamine citrate (VII), 1-p-chlorobenzyl-2-(1-pyrrolidinylmethyl)-benzimidazole HCl (Allercur) (VIII), antazoline methanesulfonate and HCl (IX), 5-benzyl-1,2,3,4-tetrahydro-2-methyl-γ-carboline (Incidal) (X), cyproheptadine HCl (XI), and oxomemazine (XII) were chromatographed.

Apparatus. Glass plates (200 × 200 mm or 200 × 100 mm); glass developing tanks lined with solvent saturated filter paper.

Adsorbent. The adsorbents were silicagel G (A_1), silicagel $HF_{254+366}$ (A_2) and Al_2O_3 basic HF_{254} (A_3) (Merck).

The plates were coated with a layer of adsorbent, 0.5 mm thick, activated at 110° for 20 minutes.

Solvent. The following developing solvents were used:

S_1: chloroform-methanol (80:20), S_2: chloroform-methanol (80:25), S_3: chloroform-methanol (80:30), S_4: chloroform-methanol (80:40), S_5: ethanol-ammonia % 25 (80:20), S_6: methyl ethyl ketone-ammonia % 25 (100:30) (organic phase), S_7: methyl ethyl ketone-chloroform-ammonia % 25 (90:10:5) (organic phase), S_8: methyl ethyl ketone-dimethylformamide (100:25).

The development time at 20°C with A_1, A_2 and A_3 respectively are for S_1 23, 27, 30; for S_2 25, 25, 25; for S_3 43, 30, 30; for S_4 45, 45, 45; for S_5 15, 20, 25; for S_6 60, 60, 60; for S_7 30, 30, 25; for S_8 30, 28, 28 minutes.

Spray reagents. Six spray reagents were used in this investigation and the color obtained with antihistamines are listed in Table I.

Table I. Spray reagents and color of spots

Spray reagents	Substances	Color
Dragendorff	all the substances	orange
Picric acid(*)	» »	yellow
Iodine-potassium iodide (on heating the chromatograms at 110°C for 10 mins.)	» »	dark brown
Modified König reagent	I to V	red
	VIII	orange
Sodium-1,2-naphtoquinon disulfonate(*)(**)	I to VII	orange
	VIII	green
	IX and X	brown
Alizarin(*)	I to VIII	yellow
	IX to X	violet

*: a saturated solution in ethanol. **: on heating the plates at 110°C for 10 mins. The color change to green with substance I, to yellow with substances II and VII, to violet with substances VIII-X.

Standards. Solutions of each substances (I-XII) in ethanol, containing appropriate amounts, were freshly prepared.

Identification

5 μl each of the samples for analysis, and an ethanolic solution of standard were applied. The plates were developed through a distance of 15 cm, dried at room temparature and sprayed with appropriate reagent (for A_1) or examined under UV light 254 mμ (for A_2 and A_3).

For substances number I-V, developing system S_1, S_2, S_3, S_5 and S_7 were used and the following solutions or pharmaceuticals were investigated:

Pheniramine maleate solution. 100 - 400 mcg/ml.

Avil ointment. The ointment diluted with ethanol to obtain a solution containing 120 mcg/ml.

Chlorpheniramine maleate solution. 100 - 400 mcg/ml.

Nostil drop. Diluted with ethanol to obtain a solution containing 100 to 400 mcg/ml.

Coryban D capsuls. 5 capsuls were extracted with ethanol, filtered and diluted to obtain a solution containing 100 to 400 mcg/ml.

Tusifon capsules. Treated as described under Coryban D capsule.

Deksan syrup. The syrup was applied directly.

Brompheniramine maleate solution. 100 - 400 mcg/ml.

Pyrilamine maleate and thonzylamine HCl solutions. 100 - 300 mcg/ml.

The reproduction of a developed plate is shown in Fig. I and Rf values in different solvents are given in Table II.

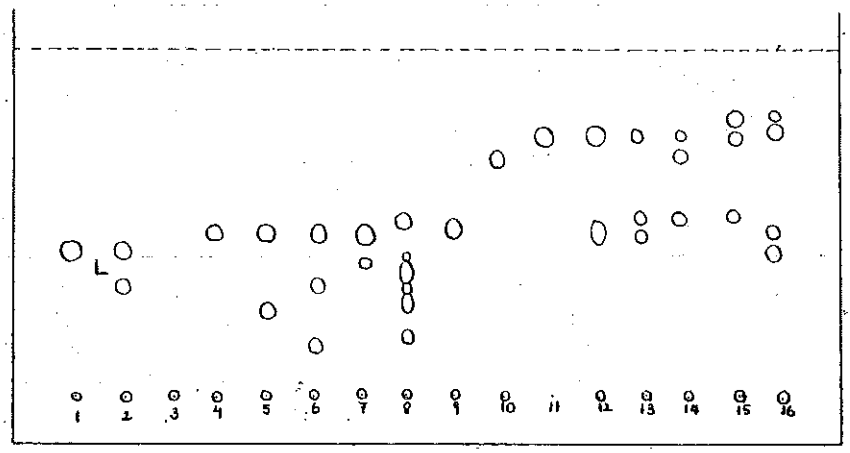

Fig. 1. (A_2 , S_7) - 1. pheniramine maleate hRf 0 and 44; 2. Avil ointment; 3. maleic acid hRf 0; 4. chlorpheniramine maleate hRf 0 and 46; 5. Nostil drop; 6. Coryban D capsules; 7. Tusifon capsules; 8. Deksan syrup; 9. brompheniramine maleate hRf 0 and 47; 10. pyrilamine maleate hRf 0 and 67; 11. thonzylamine HCl hRf 70; 12. mixt. of 1+4+11; 13. mixt. of 1+4+9+11; 14. mixt. of 4+10+11; 15. mixt. of 9+10+11; 16. mixt. 1+4+9+10+11.

For substances number VI and VII S_4 , S_6 and S_8 were used and following pharmaceuticals were investigated (Fig. 2 and table III).

Diphenhydramine solution. 400 mcg/ml.

Pediadryl drop. Diluted with ethanol to obtain a solution containing 400 mcg/ml.

Table II. Approx. hRf values of substances I - V

Substances (number)	Adsorbents and solvents											
	S_1			S_2			S_3			S_5		
	A_1	A_2	A_3	A_1	A_2	A_3	A_1	A_2	A_3	A_1	A_2	A_3
I	33	0 and 25	0 and 76	32	0 and 38	0 and 74	25	0 and 25	0 and 84		17 and 78	0 and 84
II	39		0 and 75	38	0 and 48	0 and 76	28		0 and 86	64	17 and 78	0 and 83
III	40		0 and 75	40	0 and 51	0 and 77	30		0 and 86		17 and 80	0 and 82
IV	41	0 and 42	0 and 76	40	0 and 40	0 and 76	44	0 and 42	0 and 85	64	17 and 80	0 and 82
V				62		78	54		74	67	70	83

The minumum detectable amounts were 0.5 mcg.

Benadryl elixir. Diluted with ethanol to obtain a solution containing 500 mcg/ml.

Benadryl capsules. 4 capsules were extracted with ethanol and the extract were diluted to 100 ml, 1000 mcg/ml.

Caladryl lotion. 10 g were diluted with ethanol to 50 ml, 100 mcg/ml.

Phenyltoloxamine citrate solution. 600 mcg/ml.

Bristamin lotion. 5 g were diluted with ethanol to 50 ml, 1000 mcg/ml.

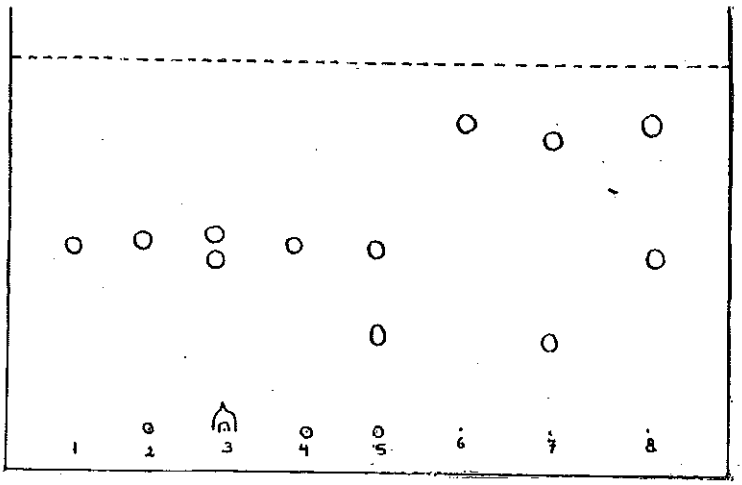

Fig. 2. (A_1, S_4) - 1. diphenhydramine HCl hRf 49; 2. Pediadryl drop; 3. Benadryl elixir; 4. Benadryl capsules; 5. Caladryl lotion; 6. phenyltoloxamine citrate hRf 81; 7. Bristaminlotion; 8. mixt. 1+2.

Table III. Approx. hRf values of substances VI and VII.

Substances (number)	Adsorbents and solvents					
	A_1	S_6 A_2	A_3	A_1	S_8 A_2	A_3
VI	76	76	90	51	48	96
VII	75	75	90	59	59	

The minumum detectable amounts were 2 mcg for VI and 32mcg for VII.

For substances VIII-X, S_2, S_3, S_5 and S_6 were used and following pharmaceuticals were investigated (Fig. 3 and Table IV).

1-p-Chlorobenzyl-2-(pyrrolidinylmethyl)-benzimidazole HCl solution. 1.5 mcg/ml.

Allercur injection. The content of 5 ampuls were diluted to 25 ml with ethanol to obtain a solution containing 2 mg/ml.

Allercur tablets. 5 tablets were extracted with ethanol to obtain a solution containing 2 mg/ml.

Antazolin HCl or methanolesulfonate solutions. 1.5 mg/ml.

Antistin tablets. 3 tablets were extracted with ethanol to obtain a solution containing 1.5 mg/ml.

Antistin injection. The contents of 3 ampuls were diluted with ethanol to 150 ml; 2 mg/ml.

5-benzyl-1,2,3,4-tetrahydro-2-methyl-γ-carbolin naphtelene-1,5-disulfonate solution. 40 mcg/ml.

Incidal tablets. 6 tablets were extracted with a solution of sodium hydroxide (5 percent), the base were extracted with chloroform, chloroform was evaporated, the residue dissolved in ethanol and diluted to obtain a solution containing 2 mg/ml.

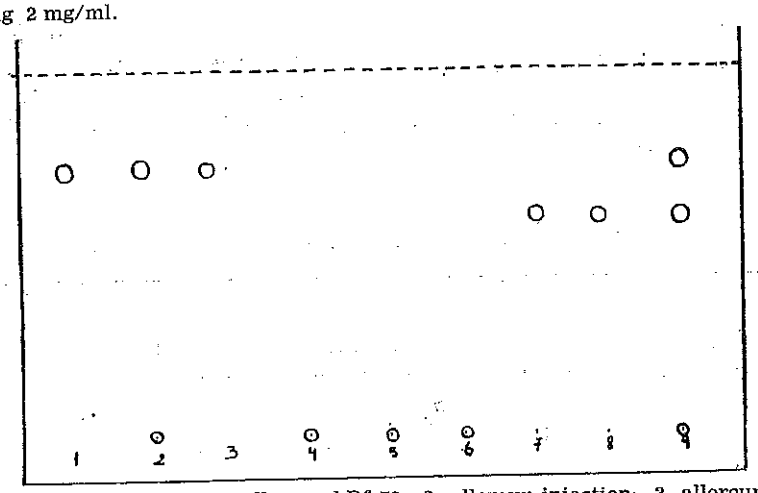

Fig. 3. (A_1, S_1) - 1. allercur hRf 73; 2. allercur injection; 3. allercur tablets; 4. antazoline HCl or methansulfonate hRf 0; 5. Antistin tablets; 6. Antistin injection; 7. Incidal hRf 60; 8. Incidal tablets; 9. mixt. of 1+4+7.

Table IV. Approx. hRf values of substances VIII - X.

Susbstances (number)	Adsorbents and solvents								
		S_2			S_5			S_6	
	A_1	A_2	A_3	A_1	A_2	A_3	A_1	A_2	A_3
VIII	75	77	92	75		81	81	83	87
IX	11	15	96	59	59	81	70	70	71
X	63	64	96	74	86	86	83	88	88

The minimum detactable amounts were 7.5 mcg for VIII and IX, 0.2 mcg for X.

Substances XI and XII chromatographed on A_1, A_2, A_3 with the solvents S_1, S_2, S_5, S_6 and S_7 and the following pharmaceuticals were investigated (Fig. 4, Table V).

Cyproheptadine HCl solution. 400 to 600 mcg/ml.

Periactin tablets. Five tablets extracted with ethanol and diluted to obtain a solution containing 400 mcg/ml.

Perideca tablets. Trated as described under Periactin tablet.

Periactin syrup. 5 ml were diluted with ethanol to 25 ml, 400 mcg/ml.

Oxomemazine solution. 100 - 200 mcg/ml.

Toplexil syrup. Diluted with ethanol to 10 ml, 100 mcg/ml.

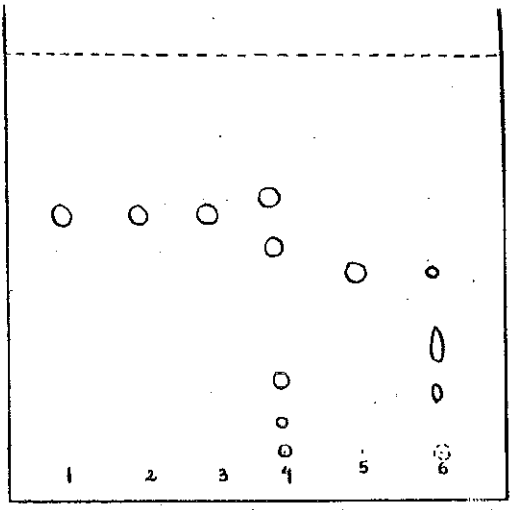

Fig. 4. (A_1, S_2) - 1.cyproheptadine HCl hRf 57; 2. Periactin tablets; 3. Perideca tablets; 4. Periactin syrup; 5. oxomemazine hRf 42; 6. Toplexil syrup.

Table V. Approx. hRf values of substances XI - XII.

Substances (number)	Adsorbents and solvents								
		S_1			S_5			S_6	
	A_1	A_2	A_3	A_1	A_2	A_3	A_1	A_2	A_3
XI	59	55	60	66	74	72	83	80	94
XII	48	53	62	60	72	78	81	80	96

The minumum detectable amounts were 2mcg for XI; 0.5 mcg for XII.

Quantitative determination

Application of drugs and development of chromatogram. Three solution of each drug were prepared: a standard, two diluted standards and the unknown (the unknown solutions were prepared from the trade preparations). The sample for analysis, a standard solution and a diluted standard solution (5 μl of each solution, 2.5, 1.25 and 0.625 mcg/ml of pheniramine maleate, 1, 0.8, 0.5 and 0.4 mcg/ml of chlorpheniramine maleate, and 3, 2, 1.5 and 1 mcg/ml of all other antihistamines) were applied to the plates by expressing 5 μl of the solution from a micrometrer syringe, in a single operation. The spot area was about 6 mm in diameter. The plates were developed using solvent S_2 (for Avil oinment S_8, for Caladryl lotion S_7 and for Antistin tablets S_6 were used), and sprayed with Dragendorff reagent.

Measurement of spot area. The plates after being developed and sprayed with Dragendorff reagent or after examined under UV light and outlined carefully with a needle were sprayed with «Neatan» (Merck) to obtain an undispering film. A sheet of transparent milimeter graph paper was superimposed on the spot, the area traced and calculated. 2, 1.5 and 1.333 dilution factors were used for diluted standards.

The values obtained were quoted in Table VI.

Table VI. Quantitative results of some pharmaceuticals.

Trade prepn.	Amount labelled mcg(per application)	Amount recovered mcg	Recovered %	Determination no.
Avil ointment	6.25	6.26 ± 0.09	100.1	28
	12.50	12.40 ± 0.21	99.2	28
Coryban D capsules	5	5.09 ± 0.42	101.8	109
	4	4.09 ± 0.13	102.2	86
Tusifon capsules	5	5.07 ± 0.41	101.4	109
	4	3.96 ± 0.13	99.0	86

Trade prepn.	Amount labelled mcg (per application)	Amount recovered mcg	Recovered %	Determination no.
Nostil drop	5	5.02 ± 0.41	100.4	109
	4	3.94 ± 0.13	98.5	86
Benadryl capsules	15	14.88 ± 0.14	99.2	196
	10	10.33 ± 0.23	103.3	79
Benadryl elixir	15	15.08 ± 0.14	100.5	196
	10	10.33 ± 0.23	103.3	79
Pediadryl drop	15	14.92 ± 0.21	99.4	196
	10	10.04 ± 0.15	100.4	79
Caladryl lotion	15	15.20 ± 0.23	101.3	26
	10	10.00 ± 0.22	100	26
Allercur injection	15	15.04 ± 0.15	100.2	123
	10	9.74 ± 0.12	97.4	59
Allercur tablets	15	15.08 ± 0.15	100.5	123
	10	10.01 ± 0.12	100.1	59
Antistin injection	15	15.11 ± 0.10	100.7	84
	10	10.02 ± 0.13	100.2	38
Antistin tablets	15	14.84 ± 0.10	98.9	29
	10	10.06 ± 0.12	100.6	65
Incidal tablets	15	15.44 ± 0.47	102.9	98
	10	9.92 ± 0.29	99.2	49
Perideca tablets	15	15.09 ± 0.07	100.6	65
	10	9.93 ± 0.08	99.3	109
Periactin tablets	15	15.01 ± 0.09	100.06	65
	10	9.93 ± 0.08	99.3	109
Oxomemazine	15	14.94 ± 0.09	99.6	67
	10	10.37 ± 0.16	103.7	34

RESULTS and DISCUSSION

Several mixtures of antihistamines were prepared to simulate trade preparations. These mixtures and the trade preparations were analyzed. Separation, identification and quantitative estimation of the antihistamines were accomplished by TLC, with appropriate developing solvent mixtures and reagents. Eight solvent systems were found to be suitable for 12 antihistamines examined and six spray reagents used for detection of the spots. Pure antihistaminic substances were used as referance standard; the drugs were identified and estimated in multicomponent preparations. The hRf values and minumum detectable amounts for each substances were determined.

Qualitative analyses. Using solvent system S_7 the following substances and their mixture were separated: pheniramine, chlor-or-brompheniramine and pyrilamine maleates and thonzylamine HCl. With the solvent system S_4, diphenhydramine HCl and phenyltoloxamine citrate were easily identified in pharmaceuticals and in their mixtures. Using solvent system S_3, allercur, antazolin salts and incidal were identified in trade preparations and were separated in the mixture. For the identification of cyproheptadine HCl and oxomemazine in the preparations S_2 was found to be the most suitable.

The chromatography of the salts of maleic and citric acids (substances No. I, II, III, IV and VI) resulted in the separation of their acids and bases component. The spots of organic acids were detected under UV light or by spraying the plates with a pH indicator such as bromphenol blue, bromcresol purple.

Quantitative analysis. With successful resolution for qualitative identification, the investigations were extended to studies the quantitative estimation of antihistamines in solution or in multicomponent preparations. The investigation fell into two parts: (1) to ascertain whether the concentration of single drug could be related directly to their spot area; for this purpose the linearity between the area and the concentration was determined with 12 pure antihistamines in three different concentrations; (2) to apply the technique to the analysis of trade preparations. In practice the area of application should be 6-8 mm diameter; with the appropriate solvent system and using Dragendorff reagent, it was possible to have a circular and obvious spot.