DRUGS, COSMETICS, AND FORENSIC SCIENCES

Rapid Identification and Quantification of Chlorpheniramine Maleate or Pheniramine Maleate in Pharmaceutical Preparations by Thin-Layer Chromatography-Densitometry

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Thin-layer chromatography (TLC)-densitometry was used to separate, identify, and quantitate chlorpheniramine maleate (CPM) and pheniramine maleate (PM) when present in combination with other drugs in pharmaceutical preparations of tablets, syrups, eye and ear drops, etc. CPM or PM was extracted (tablets, capsules, etc.) or diluted (liquid preparations, if needed) with 80% ethanol and isolated from other ingredients by TLC on silica gel G using cyclohexane–chloroform–methanol–diethylamine (4.5 + 4.0 + 0.5 + 1.0, v/v) as the mobile phase. Separated CPM and PM were detected under shortwave ultraviolet light and quantitated by scanning densitometry at 260 nm. Recoveries of CPM and PM were 100.09 ± 0.77% and 100.09 ± 0.87%, respectively.

Chlorpheniramine maleate (CPM) is an antihistaminic drug mainly used in anti-allergic and cold preparations, such as tablets, syrups, eye and ear drops, etc. These are estimated by methods involving nonaqueous titration (1, 2), spectrophotometry (3, 4), and fluorimetry (5, 6). Colorimetric methods for estimation based on the reaction with cyanogen bromide (7–9), yellow color complex formation with bromocresol green (10), and formation of the "Reinecket" salt (11, 12) have been reported. Various liquid chromatography (LC; 1, 13–15), gas chromatography (GC; 16–19), thin-layer chromatography (TLC; 20–24), and TLC/mass spectrometry (25) methods have also been reported for the determination of CPM from combined dosage forms with other drugs.

CPM or pheniramine maleate (PM) is determined by nonaqueous titration with perchloric acid using official methods (19, 26). These methods, however, are not applicable to formulations containing these drugs because of interference from other ingredients.

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There are many analytical methods for the quantitation of these drugs. However, CPM is generally assayed by Koenig's reaction (27, 28) when present in dosage forms in combination with other drugs. The general reaction involves formation of an adduct through attack on the pyridine ring by cyanogen bromide, followed by coupling the resulting compound with a suitable organic base. The most serious problem is that cyanogen bromide and potassium cyanide are violent poisons and are unpleasant to handle. Because it is difficult to observe the proper precautions when handling large number of samples, a fatal accident may happen. To alleviate these problems, we developed a TLC system that, in combination with scanning densitometry, can separate, identify, and quatitate the CPM and PM in formulations. This chromatographic method is sensitive, accurate, fast, versatile, economical, and reproducible.

Experimental

Apparatus

(**a**) *Densitometer.*—Shimadzu (Kyoto, Japan) dual-wavelength TLC Scanner Model CS 930.

(**b**) *Ultraviolet viewer.*—Desaga UVIS System (Heidelberg, Germany).

(**c**) *Sample applicator.*—Camag (Muttenz, Switzerland) Nanomat II with 1 μL micropipet.

(**d**) *Table centrifuge.*—Swing head, 5000 rpm, Model Remi R8C (Mumbai, India).

(**e**) *TLC plates.*—10 × 10 cm, cut from 20 × 20 cm precoated silica gel F254 aluminium backed TLC plates (E. Merck, Darmstadt, Germany).

(**f**) *TLC chamber*.—Camag twin trough chamber for 10 × 10 cm plates (No. 0225155).

Reagents and Chemicals

(**a**) *TLC developing solvent.*—Cyclohexane–chloroform–methanol–diethylamine (4.5 + 4.0 + 0.5 + 1.0, v/v).

(**b**) *Ethanol.*—Dehydrated ethyl alcohol (95%) was used if not stated otherwise.

(**c**) *Standard solutions*.—CPM or PM, 1.0 mg/mL in water–ethanol (1 + 4, v/v). A graded range of 0.25 to 32 mg/mL was used for the linearity study.

Sample Preparation

(**a**) *Tablets.*—Weigh 20 tablets accurately and grind to a smooth powder with a mortar and pestle. Accurately weigh powder equivalent to 5 mg CPM or PM and transfer to a 5 mL volumetric flask. Add 1.0 mL water. Disperse the powder, add 3 mL ethanol, shake thoroughly on a Vortex mixer for 2 min, make up to the mark with ethanol, centrifuge, and use the clear supernatant alcoholic solution for TLC.

(**b**) *Liquid preparations other than syrups.*—Dilute pharmaceutical preparations such as injection, pediatric drops, etc. with ethanol to produce a 0.2 mg/mL solution of CPM or PM. If any precipitate appears, remove by centrifugation, and use the clear supernatant alcoholic solution for TLC.

(**c**) *Syrups.*—Treat samples containing >3 mg/mL CPM or PM, such as liquid preparations other than syrups described in (**b**). For more dilute samples, transfer an amount equivalent to 1 mg CPM or PM to stoppered centrifuge tubes. Add 1 g ammonium sulfate/mL of syrup and mix well. Add 3 mL benzyl alcohol–ethanol (5 + 95, v/v), shake thoroughly on a Vortex mixer for 2 min, and centrifuge. Remove the alcoholic layer into a 5 mL volumetric standard flask and repeat procedure with a further 1 mL benzyl alcohol–ethanol (5 + 95, v/v). Make up the volume of the extract with ethanol, mix and centrifuge to clarify, if needed. Use of dehydrated ethanol helps to precipitate TLC-interfering sugars from liquid and syrup preparations.

For the recovery study with tablets, 0.1 mL of the corresponding standard solution (concentration 50 mg/mL) was added to the powder taken in a volumetric flask, allowed to dry, and processed as stated above. For liquid preparations, an equivalent amount of the corresponding standard solution was added to the sample before further processing.

Quantitative Analysis by TLC

For detection and quantification by scanning densitometry, a Nanomat II sample applicator was used to apply 1 μL of the test solution and 1 μL of the corresponding standard solution. For liquid preparations (**b**) and (**c**), 2 μL of the test solutions and 2 μL of corresponding standard solution (of approximately the same concentration) were applied as separate compact spots 10 mm apart and 10 mm from the bottom of the plate. The plate was developed up to 0.5 cm from the top in a filter paper lined tank saturated (1 h) with the mobile phase. After chromatography was completed, solvents were removed from the plate in a current of air, and the CPM or PM spots were visualized under shortwave UV light. For determination of standard error, 6 separate preparations of the same samples were used. Samples and standard (of equivalent concentration) were applied as separate spots on the same plate and developed. The marked lanes were scanned at 260 nm. Parameters for scanning were set according to the instruction manual of the densitometer. Linearity of a plot of integrated area vs concentration was assessed by measuring area values produced by different known amount of standard solutions of CPM and PM (graded concentrations). Standard preparations were applied as 6 separate spots on the same plate and developed. Separate plates were used for separate standard concentrations.

Table 1. Determination of chlorpheniramine maleate and pheniramine maleate by proposed method

Sample[a]	No.	Claim, mg/DU[b]	Found[c]	
			Amount, mg/DU[b]	% of claim
Chlorpheniramine maleate	1	2.0	1.97 ± 0.03	98.68 ± 1.49
	2	2.0	1.96 ± 0.04	98.10 ± 2.13
	3	4.0	4.06 ± 0.07	101.40 ± 1.74
	4	2.5	2.54 ± 0.03	101.60 ± 1.35
	5	2.0	1.99 ± 0.03	99.81 ± 1.24
Pheniramine maleate	6	2.0	2.03 ± 0.04	100.38 ± 1.35
	7	4.0	4.06 ± 0.06	101.60 ± 1.53
	8	12.5	12.34 ± 0.24	98.72 ± 1.94
	9	15.0	14.82 ± 0.15	98.83 ± 0.98

[a] Sample composition: (1) Paracetamol 500 mg, phenylpropanolamine HCl 25 mg, chlorpheniramine maleate 2 mg (per tablet); (2) phenylephedrine HCl 5 mg, chlorpheniramine maleate 2 mg, aspirin 325 mg, caffeine 30 mg (per tablet); (3) phenylpropanolamine HCl 25 mg, chlorpheniramine maleate 4 mg, paracetamol 325 mg (per tablet); (4) ephedrine HCl 6 mg, codeine phosphate 10 mg, sodium citrate 50 mg, chlorpheniramine maleate 2.5 mg, menthol 0.5 mg (per 5 mL syrup); (5) chlorpheniramine maleate 0.2%, phenylephrine HCl 0.12%, antipyrine 0.1%, methyl-*p*-hydroxybenzoate 0.1% (eye/ear drops); (6) phenylephrine HCl 2.5 mg, pheniramine maleate 2 mg, benzalkonium chloride 0.2 mg, thimersal 0.02 mg, menthol 0.25 mg, eucalyptol 0.2 mg, ethanol (95%) 0.004 mL (per mL); (7) phenylpropanolamine HCl 25 mg, pheniramine maleate 4 mg (per tablet); (8) pheniramine maleate 12.5 mg, paracetamol 500 mg (per tablet); (9) pheniramine maleate 15 mg, ammonium chloride 125 mg, menthol 1.14 mg, methyl-4-hydroxybenzoate 0.1006 mg, propyl-*p*-hydroxybenzoate 0.0118 mg (per 5 mL).

[b] DU = Dosage unit.

[c] Average of 6 independent determinations ± standard deviation (*see* text).

Table 2. Relationship between concentration of chlorpheniramine maleate or pheniramine maleate with corresponding integrated area value obtained by densitometry after TLC

	CPM area value[a]			PM area value[a]		
Amount applied, μg	Area	SD[b]	RSD[c], %	Area	SD[b]	RSD[c], %
0.25	4267	96	2.25	5455	56	1.03
0.50	8668	58	0.67	11181	130	1.16
1.00	16954	77	0.45	20420	342	1.67
2.00	32277	537	1.66	34840	180	0.52
4.00	60327	844	1.40	58985	529	0.90
8.00	115841	3046	2.63	101356	1089	1.07
16.00	203551	3669	1.80	176708	1135	0.64
32.00	361645	2241	0.62	304974	3636	1.19

[a] Average of 6 independent determinations (*see* text).
[b] SD = Standard deviation.
[c] RSD = Relative standard deviation.

Results and Discussion

Table 1 shows assay results of CPM and PM from some commercial proprietary preparations. The integrated area values showed an excellent linear relation with the concentration of CPM or PM in the test solution (0.25–8 mg/mL; Table 2). The correlation coefficient (r) was 0.9996 and 0.9958, respectively, for CPM and PM. Excellent recovery of CPM and PM were obtained when these drugs were added to laboratory-made syrup preparation. Validation of the proposed method for these samples was done by a recovery test. The percent recoveries of CPM and PM were 100.09 ± 0.77 and 100.09 ± 0.87, respectively, showing the excellent reliability and consistency of the proposed method

Table 3. Recovery of chlorpheniramine maleate or pheniramine maleate added to commercial preparations and assayed by the proposed method[a]

Sample[b]	No.	Claim	Found	Added	Total found	Recovery	Recovery, %
Pheniramin maleate	1	—[c]	—	5.00	5.00 ± 0.08	5.00 ± 0.08	100.09 ± 1.56
	2	—	—	10.00	10.01 ± 0.14	10.01 ± 0.14	100.13 ± 1.35
	3	12.50	12.34 ± 0.13	12.50	24.91 ± 0.12	12.57 ± 0.17	100.53 ± 1.33
	4	15.00	15.31 ± 0.15	15.00	30.47 ± 0.36	15.16 ± 0.36	101.03 ± 2.44
	5	4.00	4.08 ± 0.08	4.00	8.03 ± 0.11	3.95 ± 0.12	98.69 ± 3.08
Overall recovery							100.09 ± 0.87
Chlorpheniramin maleate	6	2	2.02 ± 0.05	2.00	4.00 ± 0.05	1.98 ± 0.07	99.08 ± 3.31
	7	2	1.97 ± 0.02	2.00	3.98 ± 0.07	1.98 ± 0.07	100.58 ± 3.21
	8	5	5.02 ± 0.12	5.00	10.07 ± 0.07	5.06 ± 0.13	101.07 ± 2.51
	9	2.5	2.51 ± 0.01	2.50	5.00 ± 0.03	2.49 ± 0.03	99.68 ± 1.26
	10	4	4.06 ± 0.04	4.00	8.10 ± 0.04	4.04 ± 0.05	100.04 ± 1.29
Overall recovery							100.09 ± 0.77

[a] Values as in Table 1.
[b] Sample composition: (1) bromhexine HCl 8 mg, terbutaline sulfate 4 mg, guaiphenesin 200 mg (per 10 mL syrup); (2) analgin 250 mg, paracetamol 250 mg, caffeine 25 mg (per tablet); (3) pheniramine maleate 12.5 mg, paracetamol 500 mg (per tablet); (4) pheniramine maleate 15 mg, ammonium chloride 125 mg, menthol 1.14 mg, methyl-*p*-hydroxybenzoate 0.1006 mg, propyl-*p*-hydroxybenzoate 0.0118 mg (per 5 mL syrup); (5) phenylpropanolamine HCl 25 mg, pheniramine maleate 4 mg (per tablet); (6) paracetamol 500 mg, phenylpropanolamine HCl 25 mg, chlorpheniramine maleate 2 mg (per tablet); (7) phenylephrine HCl 5 mg, chlorpheniramine maleate 2 mg, aspirin 325 mg, caffeine 30 mg (per tablet); (8) phenylephedrine HCl 5 mg, chlorpheniramine maleate 5 mg, caffeine 15 mg, paracetamol 500 mg (per tablet); (9) ephedrine HCl 6 mg, codeine phosphate 10 mg, sodium citrate 50 mg, chlorpheniramine maleate 2.5 mg, menthol 0.5 mg (per 5 mL syrup); (10) phenylpropanolamine HCl 25, chlorpheniramine maleate 4 mg, paracetamol 325 mg (per tablet).
[c] — = Sample did not contain pheniramin maleate.

Table 4. hR_f[a] ($R_f \times 100$) and hR_{ST}[b] values of some common ingredients of dosage forms containing chlorpheniramine maleate or pheniramine maleate on silica gel plates using the proposed mobile phase

Sample	hR_f	hR_{ST}
Chlorpheniramine maleate	66.3	100
Pheniramine maleate	66.3	100
Caffeine	55.8	84.1
Ephidrine	41.7	62.8
Guaiphenesin	36.3	54.7
Phenylephrin	27 (tailing)	40.7
Ibuprofane	20.1	30.3
Paracetamol	18.5	28.0
Diclofenac sodium	18.3	27.6
Codein phosphate	0	0

[a] Values are dependant on many factors and may vary considerably.

[b] hR_{ST} is (R_f of sample/R_f of chlorpheniramine maleate) × 100.

(Table 3). Extraction with ethanol–benzyl alcohol (5 + 95, v/v) showed excellent recovery of these drugs when present in liquid preparations in low concentrations. Ammonium sulfate added to the sample tube before extraction improved recovery (29). The low value of standard deviation obtained even at low concentrations of the drugs indicates high reproducibility of the proposed method.

TLC plates stored in the laboratory may show a high background noise due to absorbed laboratory fumes. Prior cleaning of such plates by developing with methanol followed by air-drying for 2 h greatly improves the chromatogram.

The results indicate the suitability of the proposed method for a variety of proprietary formulated products. The proposed method is simple, rapid, safe, and more advantageous than existing ones. Use of diethylamine in the solvent system suppresses the basic character of CPM or PM, rendering them relatively nonpolar. As such, aspirin, paracetamol, caffeine, codeine phosphate, etc., when present in the formulation, are well separated from CPM and PM and can be identified without extra cost (Table 4). These drugs may also be estimated simultaneously, provided standards of known concentration are applied side by side. We have successfully analyzed more than 100 samples by this method without any problem.

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