Research article

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Spectrofluorimetric determination of certain biologically active phenothiazines in commercial dosage forms and human plasma

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ABSTRACT: A validated simple and sensitive spectrofluorimetric method was developed for the determination of chlorpromazine hydrochloride, promethazine hydrochloride, trifluperazine hydrochloride, thioridazine hydrochloride, perazine maleate and oxomemazine. The method was based on condensation of malonic acid/acetic anhydride (MAA) under the catalytic effect of the tertiary amine moiety of the studied phenothiazines to provide a deep yellow to brown colour with green florescence. Relative fluorescence intensity of the products was measured at λ_{exc} 398 nm and λ_{em} 432 nm. Different variables affecting the reaction were studied and optimized. The method was successfully applied for the determination of the studied drugs in commercial dosage forms. The lower detection limits allowed the application of this method for the determination of the compounds in plasma as an example of a biological fluid. In addition, the method was considered specific for the determination of tertiary amines in the presence of primary and secondary amines; as a result, it was deemed suitable for the determination of the cited drugs in the presence of their degradation products resulting from N-dealkylation or oxidation of the corresponding sulphoxides or sulphones. Copyright © 2012 John Wiley & Sons, Ltd.

Keywords: spectrofluorimetric determination; phenothiazines; malonic acid; acetic anhydride; dosage forms; plasma

Introduction

Phenothiazines such as chlorpromazine hydrochloride, promethazine hydrochloride, trifluperazine hydrochloride, thioridazine hydrochloride, perazine maleate and oxomemazine are commercially available psychoactive drugs extensively used in Egypt in a variety of pharmaceutical formulations as tablets or ampoules (1). Several methods have been published for the determination of these drugs in bulk or in different pharmaceutical formulations as well as in biological fluids. These methods include volumetric methods (2–4), spectroscopy (5–13), electrochemical methods (14–16), flow injection spectrophotometry (17,18), flow injection spectrofluorimetry (19–21), chromatography (22–28), capillary electrophoresis (29) and immunoassay (30). The wide use of these drugs requires the development of simple, rapid, accurate, sensitive, applicable and less expensive methods for their determination in pure and dosage forms. As a result, this study describes a simple and very sensitive spectrofluorimetric method for the determination of these drugs based on the presence of a tertiary amine moiety. The use of a mixed anhydride system [malonic acid/acetic anhydride (MAA)] in the present work made the proposed method highly specific to the analysis of tertiary amine bases as well as amine salts without interference of primary, secondary or quaternary amines.

Experimental

Apparatus

A PerkinElmer® LS 45 luminescence spectrometer (PerkinElmer®, Waltham, MA, USA) was used connected to an IBM PC computer loaded with FL WINLAB™ software with slide width of 10 nm. A spectronic GENESYS™ 2PC Ultraviolet visible spectrophotometer (Milton Roy Co, Westhaven, USA) with matched 1-cm quartz cell connected to an IBM computer loaded with WinSpec™ application software was used for all measurements. Other instruments used included a Jenway 5056 ultraviolet–visible spectrophotometer (Bibby Scientific Limited, Staffordshire, U.K.); an MLW type thermostatically controlled water bath (Labexchange®, Burladingen, Germany); an AG 29 digital analytical balance (Meltter Toledo, Glattbrugg, Swizerland); a Milwaukee SM 101 pH meter (Milowaukee, Szeged, Hungary) and a 4000c/s laboratory centrifuge (Germany).

Materials

Pharmaceutical compounds used in this study included chlorpromazine hydrochloride and promethazine hydrochloride were supplied by Misr Co. for Pharmaceutical Industries, Alexandria, Egypt; trifluperazine hydrochloride supplied by El-Kahira Co., Cairo, Egypt; thioridazine hydrochloride supplied by Novartis Pharma S.A.E., Cairo, Egypt; perazine maleate supplied

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by Sigma-Aldrich, Cairo, Egypt; and oxomemazine supplied by Amriya Pharm Industries, Alexandria, Egypt.

Pharmaceutical formulations

The following available commercial preparations were analyzed. Phenergan® syrup (Rhone-Poulanc Rorer, Cairo, Egypt) containing 5 mg promethazine hydrochloride per 5 mL of syrup (batch no. 6218006); Neurazine® tablets (Misr Co. for Pharm Industries S. A.E., Cairo, Egypt) containing 100 or 25 mg chlorpromazine hydrochloride per tablet (batch no. B105056 for 100 mg tablet and B128029 for 25 mg tablet); Thiozine® tablets (Delta Pharmaceutical Industries, Cairo, Egypt) containing 100 or 50 mg thioridazine hydrochloride per tablet (batch no. B06901 for 100 mg tablet and B06153 for 50 mg tablet); Stellasil® tablets (Kahira Pharmaceuticals and Chem. Ind. Co., Cairo, Egypt, respectively) containing 5 or 1 mg trifluperazine hydrochloride per tablet (batch no. 0610755 for 5 mg tablet and 81435 for 1 mg tablet); Toplexil® syrup (European Egyptian Pharm. Ind., Alexandria, Egypt) containing 0.033 g oxomemazine per 100 mL syrup (batch no. 7523147); Oplex® syrup (Amriya Pharmaceutical Industries, Alexandria, Egypt) containing 0.033 g oxomemazine per 100 mL syrup (batch no. 252115); and EXE-TOP® syrup (Top Pharma Incorporation, El-Obour city, Cairo, Egypt) containing 1.65 mg oxomemazine per 5 mL syrup (batch no. 82302). All chemicals were of analytical grade. Malonic and ascorbic acids were obtained from Merck Schuchardt OHG, Hohenbrunn, Germany and citric acid and acetic acid anhydride were purchased from EL Nasr Pharmaceutical Chemicals Co., Egypt.

Experimental conditions for determination by MAA reagent

Malonic acid/acetic anhydride (MAA)10 % w/v was prepared by dissolving 5 g malonic acid in 50 mL acetic anhydride with gentile heating up to 80 °C for five min then gradually cooled to room temperature. The reagent was stable for about 10 h with a pale yellow coloration. Fifty mg of each drug was carefully transferred into 100-mL volumetric flasks and 80 mL methanol was added then sonicated until complete dissolution of the drug. The resultant solution was completed to volume with the same solvent to give a stock solution containing 500 mg/L of each drug. The solution was further diluted with the same solvent to obtain working standards covering a range of 500–6000 ng/mL.

Preparation of the standard solutions

Fifty mg of each drug was carefully transferred into 100-mL volumetric flasks and 80 mL methanol was added then sonicated until complete dissolution of the drug. The resultant solution was completed to volume with the same solvent to give a stock solution containing 500 mg/L of each drug. The solution was further diluted with the same solvent to obtain working standards covering a range of 500–6000 ng/mL.

General procedure

One ml of the working standard solution of each of the drugs was transferred into a test tube and evaporated until dry in a water bath at 80 ± 2 °C then 3 mL of 10 % w/v MAA reagent was added and mixed thoroughly. The solution was allowed to stand for 20 min at 80 ± 2 °C and then cooled to room temperature. Next, the solution was transferred to a 10-mL calibrated flask and completed to volume with ethanol. Several dilutions were then made with the same solvent to obtain overall general concentrations in the range of 5–60 ng/mL. The relative fluorescence intensities (RFI) of the resultant solutions were measured for the studied phenothiazines at $\lambda_{exc.}$ 398 nm and $\lambda_{em.}$ 432 nm against a reagent blank treated similarly.

Preparation of sample solution

Twenty tablets were weighed, finely powdered and mixed thoroughly. A weighed amount of the tablets equivalent to 50 mg of each studied drug was then transferred into a 100-mL volumetric flask and shaken thoroughly with ~ 80 mL methanol for about 10 min and then completed to volume with the same solvent. The solution was filtered and the first portion of the filtrate was rejected. A measured volume of the filtrate was further diluted with methanol to obtain working sample solutions with a final concentration of 500–6000 ng/mL. Next, the general procedure was followed as described for calibration under general procedure using the prepared sample solution instead of the standard working solutions.

A volume of promethazine HCl or oxomemazine equivalent to 5 mg was transferred to a 100-mL separating funnel contained ~ 20 mL distilled water and the aqueous layer was rendered alkaline by addition of 33% w/v aqueous ammonia solution and the liberated phenothiazine base was extracted with three volumes of 15 mL of chloroform. The chloroformic extract was filtrated through anhydrous Na_2SO_4 supported on filter paper. The obtained filtrate was evaporated to dryness using a rotary evaporator. The residue was dissolved in ~ 15 mL methanol and the resultant solution was transferred into a 50-mL volumetric flask and completed to volume with the same solvent. The final solution was diluted to obtain working standard solutions in the general concentration range of 500–6000 ng/mL. Next, the general procedure was followed as described for calibration under general procedure using the prepared sample solution instead of the standard working solutions.

General procedure for determination of the studied drugs in plasma samples

One mL of standard solution in concentration ranges of 100–450, 100–400, 100–500, 200–600, 100–450 and 100–500 mg/L of promethazine hydrochloride, perazine maleate, chlorpromazine hydrochloride, thioridazine hydrochloride, trifluperazine hydrochloride and oxomemazine, respectively, was transferred into a heparinized test tube and evaporated until dry. One mL of plasma was added and vortexed for 3 min. The mixture was then transferred into a 50-mL separating funnel and rendered alkaline through addition of 33 % w/v aqueous ammonia solution and the liberated phenothiazine base was extracted with the aid of two portions each of 3 mL dichloroethane. The organic layers were collected into a test tube and evaporated until dry on a water bath at 80 ± 2 °C. The contents were then transferred to a 10-mL calibrated flask with the aid of methanol and completed to volume with the same solvent. Several dilutions were then produced with the same solvent to obtain working standard solutions with overall general concentrations of 1000–6500 ng/mL. Next, the procedure described above was followed using the working standard solution instead of the authentic working standard solution. By the end of the procedure, several dilutions were then obtained with the same ethanol to obtain overall general concentrations in the range of 10–65 ng/mL. The relative fluorescence intensities (RFI) of the resultant solutions were measured for the final phenothiazines at λexc 398 nm and λem 432 nm against a reagent blank treated similarly.

Results and discussion

The use of a mixed anhydride system (MAA) in the present study made the developed method highly specific to the analysis of

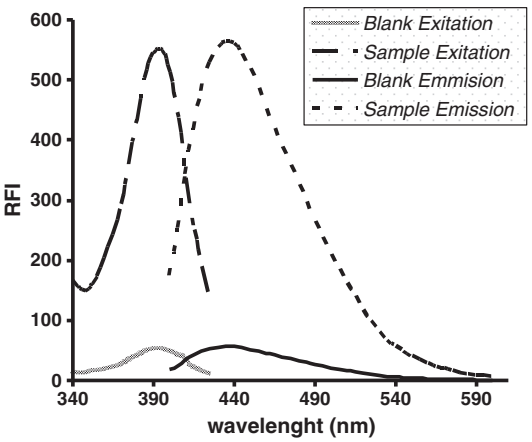

Figure 1. Fluorescence spectra of the reaction product of **MAA** with 40 ng/mL of chlorpromazine hydrochloride in ethanol.

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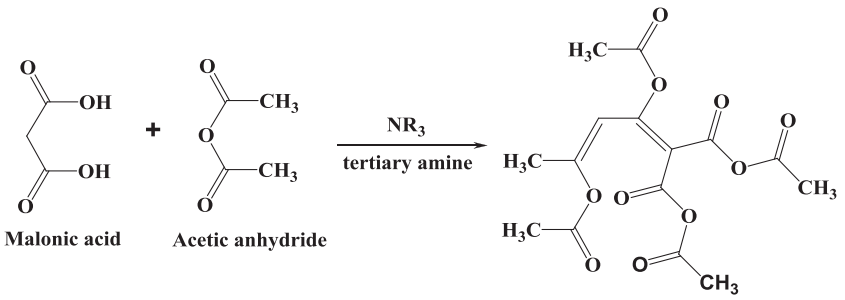

Figure 2. Condensation reaction product of malonic acid and acetic anhydride reagent under the catalytic effect of tertiary amine compounds.

tertiary amine bases as well as amine salts without interference of primary and secondary amines. All parameters affecting the fluorophore were studied. Validation of the procedure was undertaken and the method was applied successfully for the determination of the phenothiazines in pure form, pharmaceutical dosage form and in plasma.

Spectral characteristics

The spectrofluorimetric method used was based on the condensation of MAA by catalytic effect of the tertiary amine moiety of the phenothiazines. RFI of the condensation product was measured for the drugs at λ_{exc} 398 nm and λ_{em} 432 nm. Figure 1 shows the excitation and emission spectra of the fluorigenic product of chlorpromazine (as representative example of the studied drugs) with MAA reagent.

The reaction mechanism

Little is know in the literature about the mechanisms of MAA. However, it seems apparent that the final product was the result of a base-catalyzed condensation reaction of MAA where the tertiary amine groups of the phenothiazine drugs acted as a catalyst. Groth and Wallerburg (31) suggested a structure for MAA but they did not offer a definite identification about the final product (Fig. 2).

Optimization of variables

Parameters affecting RFI intensity were studied. These parameters were: effect of different acids, effect of MAA reagent concentration and volume, effect of temperature and heating time, effect of diluting solvent and finally stability of the fluorigenic product formed. In all cases, the procedure described above was followed and each parameter examined was varied while other experimental parameters were kept constant. Details of these experiments are summarized as follows.

Effect of different acids

Different acids such as malonic, ascorbic and citric acid were studied. It was found that malonic acid was the most suitable for the reaction since the RFI and stability of its condensation product was greater than that of ascorbic and citric acids whereas oxalic and tartaric acids showed high blank readings. These findings can be explained by the acidity of the $-CH_2-$ group alpha of both carboxylic groups; it

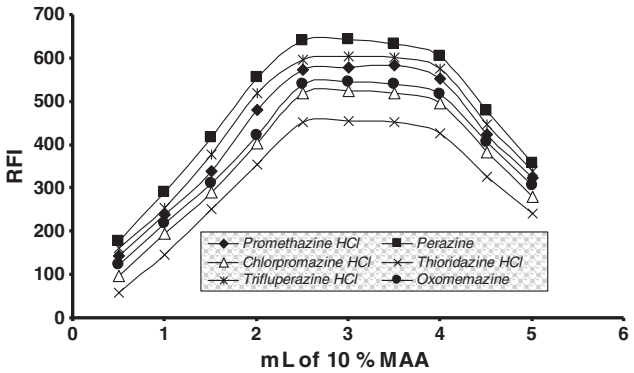

Figure 4. Effect of (10 % w/v) MAA volume on the RFI of reaction product with 40 ng/mL of the studied phenothiazine drugs.

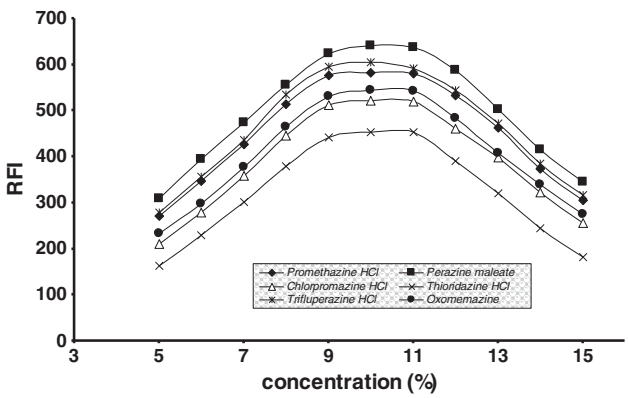

Figure 3. Effect of MAA concentrations of on the RFI of reaction product with 40 ng/mL of the studied phenothiazine drugs.

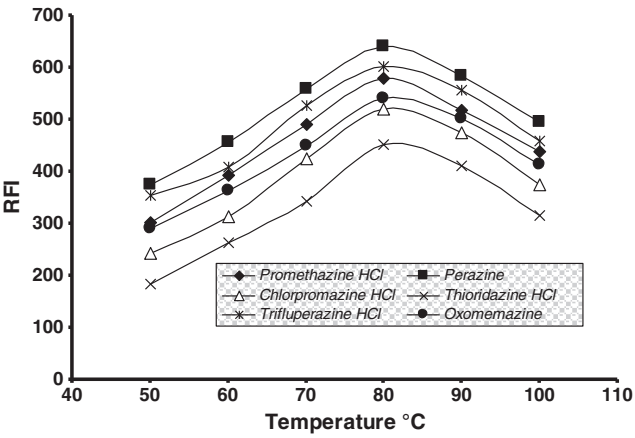

Figure 5. Effect of different temperature degrees on the RFI of the reaction product using 10 % w/v MAA with 40 ng/mL of the studied phenothiazine drugs.

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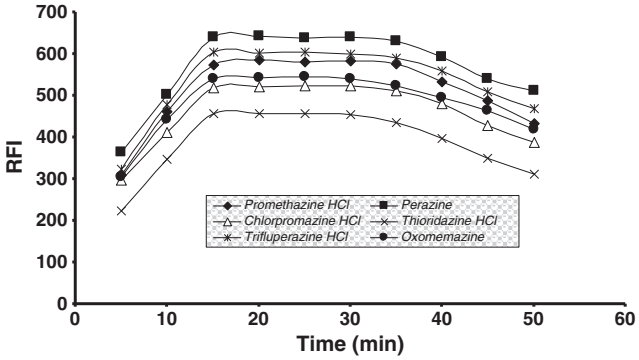

Figure 6. Effect of reaction time at 80 °C on the RFI of the reaction product using 10 % w/v MAA and 40 ng/mL of the studied phenothiazine drugs.

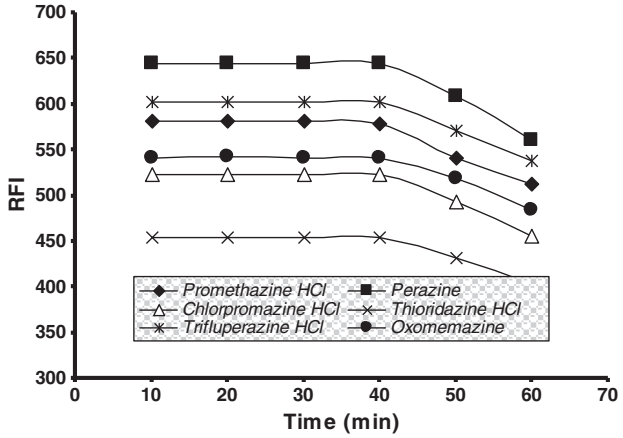

Figure 7. stability of the fluorigenic reaction product of 40 ng/mL of the studied phenothiazine drugs with 10 % w/v MAA.

facilitated condensation with acetic anhydride. Therefore, malonic acid was selected for this study.

Effect of MAA reagent concentration and volume

Results showed that RFI increased following increases in the concentration of MAA reagents until concentration levels ranged from 9-11%. As a result, 10% w/v of the reagent was selected as the optimum concentration (Fig. 3). Next, the effect of different volumes of 10% w/v of MAA reagent on RFI was studied. It was observed that RFI increased with the volume of reagent up to 2 mL and remained approximately constant up to 4 mL until a gradual decrease was observed after this volume. Consequently, 3 mL of 10 % w/v MAA reagent was selected as a suitable volume (Fig. 4).

Effect of temperature and heating time

The influence of applying different heating temperatures and incubation times were studied. A temperature at 80 ± 2 °C was found to be optimal for developing the fluorophore (Fig. 5). Complete reaction was attained at this temperature in 20 min for all studied phenothiazines (Fig. 6). These results can be explained by the fact that the condensation product was formed in a quantitative yield at the temperature and time observed. However, at elevated temperatures or at longer heating times, RFI was expected to decrease due to loss of energy through collision rather than emission.

Effect of diluent

The effect of various diluting solvents of different polarities and hydrogen bonding capacities on RFI was studied. RFI of the reaction product was significantly affected by the solvent used. Maximum RFI was obtained when ethanol was used as the diluting solvent. This could be attributed to the high solubility of the condensation product in ethanol as well as to moderate polarity. Accordingly, ethanol was chosen as the diluent for all subsequent experiments in this study.

Stability of the fluorigenic product

The stability of the reaction product as a function of time was also studied. It was found that the condensation product remained stable for about 40 min at room temperature (25 ± 2 °C) after diluting with ethanol and then a gradual decrease in RFI was observed. Therefore, all measurements throughout this study were recorded within this period (Fig. 7).

Validation of the developed method

Linearity and range

Under optimized reaction conditions, standard calibration graphs for the investigated drugs with MAA were constructed by analyzing

Table 1. Quantitative parameters using the MAA spectrofluorimetric method

Parameter	Promethazine HCl*	Perazine maleate*	Chlorpromazine HCl*	Thioridazine HCl*	Trifluperazine HCl*	Oxomemazine*
*Linear range (ng/mL)***	5-45	5-40	5-50	5-60	5-45	5-50
Intercept (a) ± SE	2.28 ± 6.76	0.22 ± 6.66	1.77 ± 5.65	0.60 ± 4.60	2.58 ± 7.23	3.48 ± 6.25
Slope (b) ± SE	14.51 ± 0.22	15.99 ± 0.23	12.98 ± 0.17	11.20 ± 0.12	15.03 ± 0.23	13.41 ± 0.18
Correlation coeff. (r)	0.9994	0.9996	0.9995	0.9996	0.9994	0.9994
LOD (ng/mL)	1.40	1.24	1.30	1.23	1.44	1.39
LOQ (ng/mL)	4.65	4.16	4.34	4.10	4.81	4.66

* λ_{exc} 398 nm, λ_{em} 432 nm
** Final dilution that was actually measured.

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Table 2. Evaluation of accuracy of the proposed MAA spectrofluorimetric method

	Taken# mg	Promethazine HCl*		Perazine maleate*		Chlorpromazine HCl*		Thioridazine HCl*		Trifluperazine HCl*		Oxomemazine*	
		Found mg	% Recovery	Found mg	% Recovery	Found mg	% Recovery	Found mg	% Recovery	Found mg	% Recovery	Found mg	% Recovery
1	20.00	19.45	97.28	20.31	101.55	20.39	101.97	19.55	97.74	20.33	101.62	20.29	101.44
2	25.00	25.21	100.83	25.25	101.02	25.23	100.93	25.36	101.44	24.59	98.38	24.78	99.12
3	30.00	30.40	101.35	30.26	100.84	29.77	99.24	30.18	100.60	30.20	100.67	30.14	100.49
4	35.00	35.34	100.99	34.69	99.11	34.62	98.90	34.91	99.75	34.67	99.06	34.34	98.11
5	40.00	39.79	99.49	39.50	98.76	39.62	99.04	40.35	100.88	39.73	99.33	40.15	100.36
Mean			99.98		100.26		100.02		100.08		99.81		99.90
RSD			1.670		1.231		1.364		1.443		1.311		1.299

Average of 3 determinations for each concentration cited.
Amount of authentic drug used for preparation of standard solution.

a series of six concentrations of each drug and taking the mean of three determinations for each concentration level and then plotting RFI versus concentrations of the drug within the specified range. Test results were established by calculation of a regression line by least squares method (32). In all cases, straight line correlation was obtained over the studied range of drug concentrations and RFI with good correlation coefficients (r). The small values of standard errors of slopes and intercepts reflected a low interfering background. Data are summarized in Table 1.

Sensitivity

The limit of detection (LOD) and limit of quantification (LOQ) for the proposed method were calculated using Equation (1, 2);

$$\mathrm{LOD} = 3.3\sigma/\mathrm{S} \quad (1)$$

$$\mathrm{LOQ} = 10\sigma/\mathrm{S} \quad (2)$$

where σ is the standard deviation of the intercept and S is the sensitivity parameters expressed by the slope of the calibration curve. LOD and LOQ of the drugs and other quantitative parameters are listed in Table 1.

Accuracy

The accuracy of the proposed method was investigated by preparing various standard solutions containing different amounts of drugs. Next, the working standard solutions were prepared followed by application of the procedure described above. Results were recorded as percent recovery $\pm$ standard deviation (Table 2). Results obtained showed close agreement between measured and true values and there were no significant differences between them with respect to accuracy.

Precision

Reproducibility was checked by five times analysis for five different concentrations of pure samples. Results are illustrated in Table 2 and agreement was observed between all concentrations chosen, indicating good reducibility of the proposed method. Inter- and intraday precisions were assessed using three different concentrations and three replicates of each concentration. The calculated relative standard deviation values were all $< 2\%$, indicating good repeatability and reliability of the methods. Results and statistical analyses are summarized in Tables 3, 4.

Interference study

An interference study was conducted by mixing known amounts of each drug with known amounts of the additive under study and the resultant mixture was subjected to the experimental procedure described above for preparation of sample solutions. It was found that the common excipients of the pharmaceutical formulations such as starch, magnesium stearate and Talc did not interfere with the results of the proposed method (Table 5). This could be explained on the basis that all additives were either non-extractable by organic solvents or behaved as non-basic compounds; as a result, they did not contribute to the reaction pathway.

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Table 3. Evaluation of the precision of the proposed MAA spectrofluorimetric method

Parameter		Promethazine HCl (% found)			Perazine maleate (% found)			Chlorpromazine HCl (% found)		
		20 mg*	30 mg*	40 mg*	20 mg*	30 mg*	40 mg*	30 mg*	40 mg*	50 mg*
Intraday	*1*	100.68	100.45	99.14	97.52	101.06	100.16	101.27	98.58	99.24
	2	101.70	99.28	100.17	98.14	98.94	99.53	101.52	101.47	100.76
	3	102.04	101.58	100.68	100.92	101.47	98.45	101.78	100.38	101.07
	Mean	101.47	100.43	99.99	98.86	100.49	99.38	101.52	100.14	100.36
	RSD	0.698	1.145	0.785	1.832	1.351	0.569	0.251	1.456	0.975
Interday	*1*	98.98	99.32	99.82	99.07	97.89	101.09	100.25	99.62	100.15
	2	100.34	100.45	101.03	99.91	98.73	100.62	100.76	101.53	100.91
	3	99.65	98.65	100.51	100.92	99.15	98.76	101.78	100.57	99.24
	Mean	99.66	99.47	100.45	99.97	98.59	100.16	100.93	100.57	100.10
	RSD	0.682	0.914	0.604	0.926	0.651	1.230	0.771	0.949	0.835

* Amount of authentic drug used for preparation of standard solution.

Table 4. Evaluation of the precision of the proposed MAA spectrofluorimetric method

Parameter		Thioridazine HCl (% found)			Trifluperazine HCl (% found)			Oxomemazine (% found)		
		25 mg*	35 mg*	45 mg*	20 mg*	30 mg*	40 mg*	25 mg*	35 mg*	45 mg*
Intraday	*1*	102.16	99.49	100.78	98.05	100.67	101.00	102.05	101.26	99.17
	2	100.35	97.72	100.98	99.02	102.01	102.00	99.70	100.21	98.67
	3	98.55	97.22	100.20	99.34	100.22	102.33	100.88	98.54	100.33
	Mean	100.35	98.14	100.65	98.80	100.97	101.78	100.88	100.00	99.39
	RSD	1.798	1.216	0.402	0.680	0.922	0.681	1.165	1.372	0.857
Interday	*1*	101.44	97.98	101.37	100.33	100.44	102.38	99.41	100.84	101.82
	2	99.64	99.49	100.97	99.67	98.43	101.34	100.59	101.88	100.83
	3	102.51	98.99	99.61	101.62	101.56	101.16	97.94	99.79	101.49
	Mean	101.20	98.82	100.65	100.54	100.14	101.63	99.31	100.84	101.38
	RSD	1.433	0.778	0.917	0.987	1.584	0.648	1.337	1.036	0.497

* Amount of authentic drug used for preparation of standard solution.

Table 5. Analysis of the investigated drugs in the presence of some common excipients using the proposed spectrofluorimetric method

Excipients	Amount added µg/mL	% Recovery ** ± SD					
		Promethazine HCl*	Perazine maleate*	Chlorpromazine HCl*	Thioridazine HCl*	Trifluperazine HCl*	Oxomemazine*
Starch	50	99.21 ± 0.33	99.01 ± 0.53	100.01 ± 0.23	100.62 ± 0.19	98.12 ± 0.45	98.99 ± 0.20
Mg stearate	50	98.99 ±1.31	100.21 ± 0.54	99.45 ± 1.25	101.58 ± 1.61	99.86 ± 0.33	101.62 ± 1.07
Talc	50	98.25 ± 0.39	100.01 ± 1.01	99.05 ± 0.86	100.28 ± 0.89	99.89 ± 0.49	99.19 ± 0.72

* Drug concentration used after final dilution (40 ng/mL).
** Average of 3 determinations ± SD.

Robustness

In this study, the robustness was thoroughly checked during the development phase by evaluating the influence of small variations in the experimental variables on the analytical performance of the method. The obtained recoveries and standard deviations indicated that small variations in the variables did not significantly affect the results of the procedure (Table 6). This

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Table 6. Robustness of the proposed MAA spectrofluorimetric method

Variation	%Recovery ± SD*					
	Promethazine HCl**	Perazine maleate**	Chlorpromazine HCl**	Thioridazine HCl**	Trifluperazine HCl**	Oxomemazine**
No variation***	99.83 ± 0.86	101.29 ± 0.795	98.47 ± 0.765	98.53 ± 0.635	101.55 ± 0.792	100.18 ± 0.910
1 MAA conc. 10% w/v MAA						
9.8%	98.62 ± 0.97	99.94 ± 1.441	98.92 ± 1.119	98.61 ± 1.353	100.56 ± 1.180	98.83 ± 0.869
10.2%	99.08 ± 1.03	99.12 ± 0.706	101.21 ± 0.773	99.53 ± 1.766	99.55 ± 0.921	101.58 ± 0.759
2 MAA volume 3 mL						
2.8 mL	99.08 ± 1.14	98.91 ± 0.866	100.06 ± 1.155	101.91 ± 0.916	99.11 ± 0.752	100.60 ± 1.003
3.2 mL	100.06 ± 0.87	99.59 ± 0.735	100.63 ± 0.862	100.01 ± 1.225	100.83 ± 0.884	101.51 ± 0.935
3 Temperature 80 °C						
78 °C	100.80 ± 0.43	99.02 ± 0.951	100.06 ± 1.151	98.53 ± 0.707	98.72 ± 0.787	100.30 ± 1.005
82 °C	98.97 ± 0.51	100.36 ± 1.002	99.86 ± 0.981	101.98 ± 1.374	99.94 ± 0.677	101.27 ± 0.550
4 Reaction time 20 min						
18 min	100.51 ± 1.03	100.51 ± 0.935	100.95 ± 1.514	99.41 ± 0.992	101.06 ± 0.821	99.09 ± 0.659
22 min	99.66 ± 0.79	98.86 ± 0.944	99.87 ± 1.303	101.17 ± 1.545	100.50 ± 0.883	100.58 ± 1.274

* Average of three determinations.
** Drug concentration used after final dilution (40 ng/mL).
*** No variation in the assay conditions of the proposed method.

assessment provided an indication of the reliability of the proposed method during routine work.

Application to pharmaceutical dosage forms. The proposed method was successfully applied for the analysis of pharmaceutical dosage forms of the investigated drugs and results obtained were validated by comparison with those of reported methods (33–35) using Student's t-test and variance ratio F-test (36,37) at 95% confidence level and showed good levels of precision and accuracy Table 7.

Recovery of the proposed spectrofluorimetric methods. Experiments were conducted to evaluate the accuracy of the proposed method on both pure authentic and dosage forms. Different concentrations of each of the authentic drugs were added to the sample preparation, which was then analyzed for

Table 7. Comparison between the proposed MAA spectrofluorimetric and reported methods for the determination of the studied phenothiazine drugs in their pharmaceutical dosage forms

Pharmaceutical dosage forms	% Recovery ± SD*		t-value	F-value
	Proposed method	Reported method**		
Chlorpromazine HCl				
Neurazine Tablets (100 mg)	99.35 ± 1.55	99.85 ± 1.03	0.49	2.25
Neurazine Tablets (25 mg)	100.12 ± 1.64	99.27 ± 0.89	0.81	3.39
Thioridazine HCl				
Thiozine Tablets (100 mg)	98.93 ± 1.46	100.35 ± 0.74	1.65	3.90
Thiozine Tablets (50 mg)	100.66 ± 1.62	99.64 ± 1.04	0.96	2.43
Trifluperazine HCl				
Stellasil Tablets (5 mg)	100.37 ± 0.78	99.49 ± 0.43	1.751.7	3.21
Stellasil Tablets (1 mg)	99.74 ± 1.28	99.46 ± 0.97	0.32	1.74
Promethazine HCl				
Phenergan® Syrup (5 mg/5 mL)	98.45 ± 1.14	97.51 ± 0.63	1.29	3.32
Oxomemazine				
Toplexil® Syrup (0.033 g/100 mL)	99.30 ± 1.24	98.97 ± 0.58	0.43	4.53
Oplex® Syrup (0.033 g/100 mL)	99.83 ± 1.02	99.46 ± 1.38	0.44	1.83
EXE-Top® Syrup (1.65 mg/5 mL)	100.34 ± 0.83	100.19 ± 1.31	0.20	2.49

* Average of five determinations.
Tabulated values at 95% confidence limit were t = 2.306, F = 6.338.
** References 33, 34, 35

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Table 8. Analysis of pharmaceutical preparations using MAA spectrofluorimetric methods by applying standard addition technique

Pharmaceutical dosage forms	Added (mg)*	% Recovery ± SD**
Chlorpromazine HCl		
Neurazine tablets (100 mg)	20.00	99.20 ± 1.14
	30.00	99.31 ± 1.29
	40.00	100.12 ± 1.19
Neurazine tablets (25 mg)	5.00	100.61 ± 1.01
	10.00	98.66 ± 0.95
	15.00	99.27 ± 1.52
Thioridazine HCl		
Thiozine tablets (100 mg)	20.00	99.03 ± 1.20
	30.00	101.06 ± 1.09
	40.00	99.69 ± 1.59
Thiozine tablets (50 mg)	15.00	100.35 ± 1.26
	20.00	98.11 ± 0.99
	25.00	99.69 ± 1.04
Trifluperazine HCl		
Stellasil tablets (5 mg)	1.00	100.80 ± 0.66
	2.00	100.63 ± 1.41
	3.00	100.53 ± 1.46
Stellasil tablets (1 mg)	0.10	98.40 ± 0.68
	0.20	98.26 ± 0.75
	0.30	99.20 ± 1.14
Promethazine HCl		
Phenergan® Syrup (5 mg/5 mL)	1.00	98.73 ± 0.66
	2.00	99.14 ± 0.66
	3.00	99.35 ± 0.96
Oxomemazine HCl		
Toplexil® Syrup(1.65 mg/5 mL)	0.50	98.72 ± 1.03
	0.75	99.78 ± 1.25
	1.00	99.71 ± 0.79
Oplex® Syrup (1.65 mg/5 mL)	0.50	99.67 ± 1.21
	0.75	99.89 ± 1.39
	1.00	99.52 ± 1.31
EXE-Top® Syrup (1.65 mg/5 mL)	0.50	99.89 ± 1.14
	0.75	98.58 ± 1.49
	1.00	100.15 ± 116

* Amount mixed of authentic drug with pharmaceutical formulation and subjected to sample preparation.
** Average of five determinations.

Table 9. Quantitative parameters of the studied phenothiazine drugs in plasma sample using the MAA spectrofluorimetric method

Parameter	Promethazine HCl*	Perazine maleate*	Chlorpromazine HCl*	Thioridazine HCl*	Trifluperazine HCl*	Oxomemazine*
*Linear range (ng/mL)***	10-45	10-45	10-55	10-65	10-45	10-50
(a)	3.299	3.172	2.800	2.716	3.520	2.962
(b)	13.643	14.277	11.664	9.697	13.514	12.342
(r)	0.9992	0.9993	0.9992	0.9991	0.9993	0.9993
LOD (ng/mL)	1.827	1.690	1.886	1.983	1.703	1.765
LOQ (ng/mL)	5.481	5.071	5.656	5.949	5.110	5.296

* λ_{exc} 398 nm, λ_{em} 432 nm.
** Final dilution that was actually measured.

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Table 10. Application of the proposed method for the determination of phenothiazines in spiked human plasma using the MAA spectrofluorimetric method

Drug	Spiked conc. (mg/L)*	Expected conc. after dilution (ng/ml)	Measured conc. after final dilution (ng/mL)	% Recovery ± SD**
Promethazine HCl	100	10	9.80	98.00 ± 1.78
	200	20	19.99	99.93 ± 1.51
	250	25	24.59	98.37 ± 1.65
	350	35	34.81	99.46 ± 1.66
	450	45	44.39	98.64 ± 0.64
Perazine maleate	100	10	10.03	100.32 ± 1.66
	200	20	20.11	100.55 ± 1.26
	300	30	29.56	98.52 ± 0.98
	350	35	34.90	99.71 ± 0.71
	400	40	39.58	98.95 ± 0.92
Chlorpromazine HCl	100	10	9.88	98.82 ± 1.53
	200	20	19.69	98.47 ± 1.26
	300	30	29.84	99.49 ± 1.28
	400	40	40.17	100.43 ± 1.15
	500	50	49.77	99.55 ± 1.48
Thioridazine HCl	200	20	20.20	101.01 ± 1.87
	300	30	29.81	99.39 ± 1.06
	400	40	39.87	99.69 ± 1.31
	500	50	49.98	99.96 ± 1.18
	600	60	59.03	98.39 ± 0.68
Trifluperazine HCl	100	10	9.97	99.71 ± 1.81
	200	20	19.71	98.54 ± 1.59
	250	30	29.85	99.51 ± 1.02
	350	35	34.94	99.85 ± 1.28
	400	40	40.08	100.19 ± 0.91
Oxomemazine	100	10	10.11	101.11 ± 1.64
	200	20	20.40	102.00 ± 1.94
	300	30	29.90	99.68 ± 1.41
	400	40	40.13	100.32 ± 0.94
	500	50	49.06	98.13 ± 1.15

* Amount of authentic drug mixed with plasma then subjected to sample preparation.
** Average of five determinations.

total amount of drug present. The difference between analytical results of the samples with and without the added drug provided the recovery of the amount of added drug (36, 37) (Table 8). Results clearly showed the accuracy of the proposed methods for specific determination of the investigated drugs without interference from common excipients.

Assay of the studied phenothiazines in plasma using MAA spectrofluorimetric method. We used plasma as a representative matrix for the studied drugs and determined, linearity range and other quantitative parameters for all drugs in plasma using the procedure described above. Data are summarized in Table 9. The re-determination of these parameters under these conditions was important for obtaining the right information about the effect of plasma as a matrix on the experimental parameters required to calculate the concentrations of the drugs. Accordingly, straight line equations were evaluated under this condition. Table 9 shows more accurate results following calculation of drug concentrations (Table 10) than using the equations provided in Table 1. The high sensitivity attained by the proposed MAA spectrofluorimetric method allowed for the determination of the studied phenothiazines in human plasma. As a result, this method was used for the assays of the drugs in human plasma. Excellent recoveries were obtained at five concentration levels of each drug covering the specified range in plasma (Table 10).

Conclusions

In this study, a simple, sensitive and precise spectrofluorimetric method was developed. The lower detection limits calculated for the fluorimetric method using MAA allowed for the application of this method for the determination of the cited compounds in plasma as an example of a biological fluid. In addition, this method was considered specific for determination of tertiary amine in the presence of primary and secondary amines. Therefore, this method could be suitable for the determination of drugs in the presence of their degradation products resulting from N-dealkylation.

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