ORIGINAL ARTICLE

SPECTROPHOTOMETRIC METHODS FOR THE SIMULTANEOUS ANALYSIS OF MECLEZINE HYDROCHLORIDE AND PYRIDOXINE HYDROCHLORIDE IN BULK DRUG AND PHARMACEUTICAL FORMULATIONS

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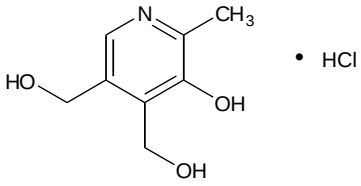
ABSTRACT

Three new spectrophotometric procedures for the simultaneous determination of pyridoxine hydrochloride and meclezine hydrochloride are described. The first method depends on the application of simultaneous equation to resolve the interference due to spectral overlapping. The analytical signals were measured at 231 and 220 nm. Calibration graphs were established for 1 to 20 μGmL^{-1} for pyridoxine hydrochloride and 0.5 to 10 μGmL^{-1} for meclezine hydrochloride in binary mixture. In the second method, the determination of pyridoxine hydrochloride and meclezine hydrochloride was performed by measuring the absorbances at 290 and 235 nm in the simple absorbance spectra of their mixture. In third method a yellowish orange complex of pyridoxine hydrochloride was formed with ferric chloride, which absorbs in the visible region with λ_{max} at 445 nm. Calibration curve of complex formation range was conducted in between 20 to 250 μGmL^{-1}. These methods were validated with respect to accuracy, precision, linearity, limit of detection and quantification. Regression analysis of Beer's plot showed good correlation in a general concentration range of 1 to 20 μGml^{-1} with correlation coefficient (r= 0.9999 and 0.9999; CV $\leq$ 0.858) for pyridoxine hydrochloride, whereas meclezine hydrochloride concentration range 0.5 to 10 μGmL^{-1} with correlation coefficient (r= 0.9998 and 0.9998; CV $\leq$ 0.826). These methods can be readily applied, without any interference from the excipients. The suggested procedures were successfully applied to the determination of these compounds in synthetic mixtures and in pharmaceutical preparations, with high percentage of recovery, good accuracy and precision.

Keywords: Ultraviolet spectrophotometry; meclezine hydrochloride; pyridoxine hydrochloride; pharmaceutical analysis; simultaneous determination, ferric chloride

INTRODUCTION

Pyridoxine hydrochloride, (5-hydroxy-6-methylpyridine-3,4-dimethyl)dimethanol hydrochloride is a white crystalline powder (structure 1), used in treatment of sideroblastic anaemias; it is readily absorbed from the gastrointestinal tract following oral administration and is converted to the active forms, pyridoxal phosphate and pyridoxamine phosphate, which are stored mainly in the liver where there is oxidation to 4-pyridoxic acid and other metabolites that are excreted in the urine. It is involved in amino acid as well as carbohydrate and fat metabolism. It is used in a variety of disorders, including the treatment of depression and other symptoms (The Merck Index 2001, Martindale, 1996, British Pharmacopoeia, 2003).



Structure 1

Meclezine hydrochloride a 1-[(RS-4chlorophenyl) phenylmethyl]-4-(3-methylbenzyl)piperazine dihydro-chloride, is yellow or yellowish-white, crystalline powder (structure 2). This piperazine derivative is antihistamine with antimuscarinic and central sedative properties. It is used in the prevention and treatment of nausea and vomiting associated with variety of conditions including motion sickness and for the symptomatic treatment of vertigo caused by Meniere's disease and other vestibular

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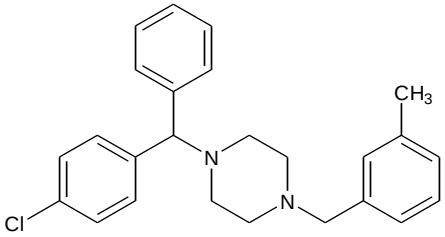
disorders. It is also used for the symptomatic relief of hypersensitivity reaction and pruritic skin disorders. It is mainly used for its antiemetic action (The Merck Index 2001, Martindale, 1996, British Pharmacopoeia, 2003).



Structure 2

Pyridoxine hydrochloride commonly used in prophylaxis and treatment of vitamin B_6 deficiency is also used in the management of nausea and vomiting of pregnancy and irradiation sickness along with doxylamine (Dorland 1994) and meclezine hydrochloride (Navidoxine, 2007). Meclezine hydrochloride was previously thought to be teratogenic, studies have demonstrated its safety during pregnancy (Miklovich and Berg, 1976).

A number of procedures for analytical quality control of pharmaceutical preparations containing pyridoxine hydrochloride alone or together with other antihistamine drugs are reported such as HPLC (Chen and Wolf, 2007; Ayi *et al*., 1986; Botticher and Botticher, 1987; Vander *et al*., 1989; Iwase, 1992; Chase *et al*., 1983; Albala *et al*., 1997; Argekar and Sawant 1999; Moreno and Salvado, 2000; Ramos *et al*., 2001; Markopoulou *et al* 2002; Vinas *et al* 2003; Sena *et al*., 2004; Marszall *et al*., 2005; Zafra *et al*., 2006; El-gindy *et al*., 2004a; Jedlicka and Klimes, 2004; El-gindy, *et al.*, 2004b; Heudi, *et al*., 2005; Hadjmohammadi *et al*., 2004; Chatzimichalakis *et al*., 2004; Monferrer *et al*., 2003), capillary electrophoresis (Fotsing *et al*., 1997) planar HPLC (Aranda and Morlock, 2006) and UV spectrophotometric (Surmeian 1998; Ayora 2000; Dinc and Baleanu, 2003; Ozdemir and Dinc, 2004). UV spectrophotometric methods were based on derivitization (Miao-Lin *et al*., 1995) while method reported for estimation of meclezine hydrochloride had limited applications (Mirza *et al*., 1999). The pyridoxine hydrochloride and meclezine hydrochloride mixture is not yet official in any Pharmacopoeia, while many such combinations are available in the local market.

The aim of this study was to develop an easy, inexpensive, accurate and least time consuming spectrophotometric methods for the simultaneous determination of pyridoxine hydrochloride and meclezine hydrochloride in raw form and from pharmaceutical formulations. In present paper, we reported three methods for the simultaneous quantitation of pyridoxine hydrochloride and meclezine hydrochloride from bulk drug and pharmaceutical formulations. In the first method, UV spectrophotometric measurements were carried out at 220 and 231 nm which are λ_{max} for meclezine hydrochloride and pyridoxine hydrochloride respectively. Both the components were quantitated using simultaneous equation to resolve the interference of each component over the other due to spectral overlapping. The limit of quantification (LOQ) values for pyridoxine hydrochloride were 1-20 μGml^{-1}, while for meclezine hydrochloride were 0.5-10 μGml^{-1}.

In the second method, the determination of meclezine hydrochloride and pyridoxine hydrochloride were carried out by measuring the absorbances at 235 and 290 nm in the simple absorbance spectra where no interference of each component over the other was observed.

In the third method, a yellow orange complex of pyridoxine hydrochloride was prepared by reacting it with ferric chloride which absorbed in the visible region of spectrum at 445 nm. Meclizine was quantitated in UV region by direct measurement of UV absorption at 235 nm and substracting the absorbance of pyridoxine. All these methods are quick, easy to follow, inexpensive, least time consuming and convenient for adoption by quality control laboratories and pharmaceutical industries.

EXPERIMENTAL

Apparatus

UV–visible double beam spectrophotometer (Shimadzu 1601) possessing a fixed slit width (2 nm) with quartz cells of 10 mm path length was used. The spectrophotometer was coupled with a P-IV computer loaded with Shimadzu UVPC version 3.91 software and a HP Laser Jet 1200 printer.

Materials

Meclezine hydrochloride and pyridoxine hydrochloride were kindly supplied by UCB (Belgium). They were tested for purity by their melting point and IR spectra (not shown). Ferric chloride was purchased from Merck Marker Pakistan. Commercial pharmaceutical formulation navidoxine tablets containing 25 mg meclezine hydrochloride and 50 mg pyridoxine hydrochloride manufactured by AGP (Private) Limited Karachi were obtained from local Pharmacies, which had an expiry of not less then 365 days at the time of study. All other chemical and reagents were of analytical grade and deionized water was used throughout these experiments.

Analysis of tablets

Average mass of 20 tablets was powdered and an amount of powder equivalent to 100 mg of pyridoxine hydrochloride was transferred to a 100 ml volumetric flask and the volume was adjusted to the mark with 0.1N hydrochloric acid. The solution was sonicated for 30 minutes and a portion of the solution was filtered and clear filtrate was diluted with 0.1 N hydrochloric acid to

obtain desired concentrations. For analysis of tablets by complex formation, the powdered tablets were dissolved in deionized water and proceeded as described later.

Preparation of standard solutions

Standard stock solutions of meclezine hydrochloride and pyridoxine hydrochloride (100 μGmL^{-1}) were prepared separately in 0.1 N hydrochloric acid. A validation set consisting of seven concentrations in working range of 1 to 20 μGml^{-1} for pyridoxine hydrochloride and 0.5 to 10 μGml^{-1} for meclezine hydrochloride were freshly prepared and scanned in the UV region (figure 1). Absorbances were measured at 231, 220, 235 and 290 nm wavelength and plotted against concentration, which followed Beer & Lambert's law. An appropriately diluted solution of dosage form in 0.1 N hydrochloric acid was also proceeded in the same way.

METHODS

Simultaneous equation method

The method is based on linear relationship between the absorbency ratio value of a binary mixture and the relative concentration of such a mixture. The quantification analysis of pyridoxine hydrochloride and meclezine hydrochloride and in binary mixture was performed by using the equations:

$Ap = a_{\lambda 1} b C_A + a_{\lambda 2} b C_B$ and $Am = a_{\lambda 1} b C_A + a_{\lambda 2} b C_B$

Where, Ap and Am denotes the absorbances of the mixture solution measured at λ_1 and λ_2 (220 and 231nm), C_A and C_B were concentrations of pyridoxine hydrochloride and meclezine hydrochloride respectively, $a_{\lambda 1}$ and $a_{\lambda 2}$ were absorptivities at isoabsorptive point.

Simple absorbance method

In this method different concentrations of pyridoxine hydrochloride were analysed at 290 nm as meclezine hydrochloride does not absorb at this wavelength, while meclezine hydrochloride was analysed at 231 nm. At these wave lengths, calibration curves were constructed and pyridoxine hydrochloride and meclezine hydrochloride were analyzed.

Complex formation method

Standard stock solution of pyridoxine hydrochloride 1000 μGml^{-1} was prepared in deionized water and diluted to a working range of 20 to 250 μGmL^{-1}. These solutions were reacted with ferric chloride (3 ml ferric chloride/25 ml of drug) and a yellowish orange complex was formed at room temperature, which was scanned in visible region against the reagent blank. Molar absorptivity of pyridoxine-Fe complex was calculated. Similarly, by the preparation of this complex, pyridoxine hydrochloride was determined in the tablet formulation.

RESULTS AND DISCUSSION

Several methods were reported for the individual and mixture determination of analytes through HPLC and spectrophotometry. (Suresh *et al*., 1989) reported a simultaneous spectrophotometric method in which meclezine hydrochloride, pyridoxine hydrochloride and caffeine were assayed. The method was based on an extension of Vierordt's method. (El Gindy *et al*., 2003) reported a method in which the pyridoxine hydrochloride together with either metoclopramide hydrochloride or meclezine hydrochloride were determined simultaneously by using graphical (second derivative of the ratio spectra) and numerical spectrophotometric methods (principal component regression and partial least squares, PLS-1 and PLS-2, applied to the zero order UV spectra of the mixture).

This analytical method was developed for assaying pyridoxine hydrochloride and meclezine hydrochloride in therapeutic and overdose concentrations range. In this report, three new methods, simultaneous equation method, simple absorbance method and complex formation method with optimum experimental parameters for each method are described. These proposed methods can be used for the determination of both analytes in synthetic mixtures and pharmaceutical preparations.

Simultaneous equation method

In this method, both components were quantitated using simultaneous equation to resolve the interference due to spectral overlapping of two components. The analytical signals were recorded at 231 and 220 nm. Regression analysis for the simultaneous equation of UV spectrophotometric method was carried out (table 1) and the linearity of the calibration graph and adherence of the method to Beer's law was validated. Quantitative determination of pyridoxine hydrochloride and meclezine hydrochloride in tablets using proposed method was performed and the results were in good agreement with the labeled amount of pyridoxine hydrochloride and meclezine hydrochloride (table 1). Closeness of the amount found to the labeled amount and the low coefficient of variation value showed that the proposed method was accurate and precise.

Simple absorbance method

In this method reference standards of pyridoxine hydrochloride and meclezine hydrochloride were scanned in UV region (fig. 1). Meclezine hydrochloride showed no absorbance at 290 nm, while pyridoxine hydrochloride showed considerable absorbance (fig. 1). Pyridoxine hydrochloride was quantitated at 290 nm from reference solution as well as pharmaceutical formulation (Navidoxine® tablet) and meclezine hydrochloride was determined at 235 nm. Table 2 summarizes relevant information of the calibration system, including its figures

of merit. It was simple, rapid and sensitive for the simultaneous determination of pyridoxine hydrochloride and meclezine hydrochloride in pharmaceutical formulation. Spectra of pyridoxine hydrochloride, meclezine hydrochloride and navidoxine® tablet are shown in figs. 1 and 2. Regression analysis of pyridoxine hydrochloride for the simple absorbance UV spectrophotometric method was carried out and the linearity of the calibration graph and adherence of the method to Beer's law was validated by high value of the correlation coefficient (*r*=0.9999). Quantitative determinations of pyridoxine hydrochloride and meclezine hydrochloride in tablets were in good agreement with the labeled claim (table 2). In addition, coefficient of variation (CV) for the determination of pyridoxine hydrochloride and meclezine hydrochloride were $\leq$ 0.858 and $\leq$ 0.826 respectively. Closeness of the amount found to the labeled amount and the low coefficient of variation value showed that the proposed method was accurate and precise.

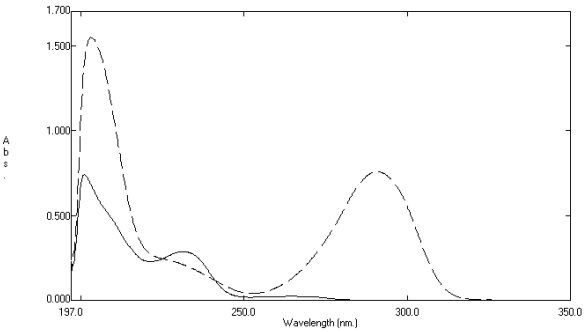


Fig. 1: Representative spectra of pyridoxine hydrochloride (---) and meclizine hydrochloride (—)

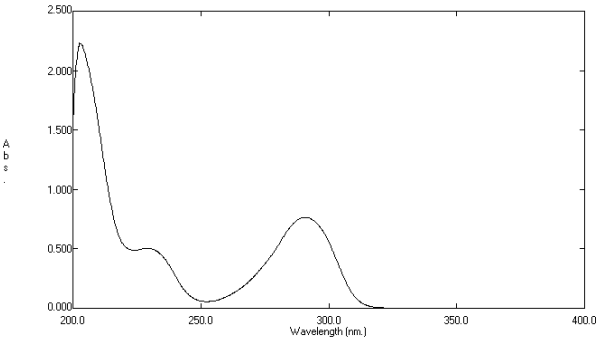


Fig. 2: Representative spectra of navidoxine tablet

Complex formation method

The complex of pyridoxine hydrochloride with ferric chloride showed absorbance in the visible region of spectrum with λ_{max} at 445 nm. This complex followed Beer's law in the concentration range of 20-250 μGml^{-1} .A representative spectra of pyridoxine hydrochloride-Fe complex is shown in fig. 3. The complex was stable at room temperature for more than 24 hours.

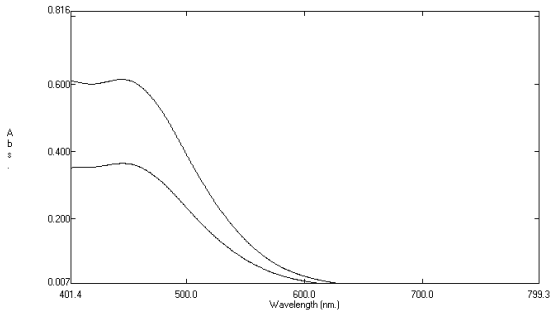


Fig. 3: Representative spectra of pyridoxine hydrochloride ferric complex in reference drug (upper line) and pharmaceutical formulation (lower line)

It was further observed that neither meclezine hydrochloride nor excipients of the tablet interfered with the reaction between pyridoxine hydrochloride and ferric chloride. Hence, initially in this method pyridoxine hydrochloride was determined in dosage formulation by complex formation and afterward simple absorbance method was used for the analysis of meclezine hydrochloride. Regression analysis of pyridoxine hydrochloride-Fe complex formation (table 3), and linearity of calibration graph was validated by high value of correlation coefficient (*r*=0.9995). Quantitative determination of pyridoxine hydrochloride in tablets using this spectrophotometric method was performed and the results were in good agreement with the labeled amount of pyridoxine hydrochloride and meclezine hydrochloride. In addition, coefficient of variation (CV) for the determination of pyridoxine hydrochloride and % recovery showed that the proposed method was accurate, precise and reliable.

Pyridoxine hydrochloride binds to ferric chloride, was first observed by Mia-lin *et al* (1995). They showed that pyridoxal reacts with –OH group and follows a second order rate constant. They were however, unable to propose the structure of Fe-complex.

METHOD VALIDATION

Linearity

The methods were validated according to International Conference on Harmonization Q2B guidelines for validation of analytical procedures (ICH 1996) in order to determine the linearity, sensitivity, precision and accuracy for each analyte. In this method the quantitation range was between 0.5-10 μGmL^{-1} for the analysis of meclezine hydrochloride and 1-20 μGmL^{-1} for the analysis of pyridoxine hydrochloride. These solutions were quantitated at 235 and 290 nm by simple absorbance method whereas at 220 and 230 nm in case of

Table 1: Recovery of pyridoxine hydrochloride and meclizine hydrochloride in pharmaceutical formulation through simultaneous equation.

Added conc. (μGml^{-1})	Found conc. (μGml^{-1})	Percentage Recovery	Added conc. (μGml^{-1})	Found conc. (μGml^{-1})	Percentage Recovery
1	1.05	104.94	0.5	0.53	105.25
4	3.99	99.69	2	1.98	99.09
8	8.14	101.75	4	3.91	97.65
10	9.81	98.14	5	4.89	97.73
12	11.89	99.05	6	5.87	97.79
16	15.81	98.85	8	7.81	97.65
20	19.60	97.98	10	10.23	102.26

Table 2: Recovery of pyridoxine hydrochloride at 290 nm and meclizine hydrochloride at 235 nm in pharmaceutical formulation through simple absorbance method.

Added conc. (μGml^{-1})	Percentage recovery	Found conc. (μGml^{-1})	Added conc. (μGml^{-1})	Percentage recovery	Found conc. (μGml^{-1})
1	101.232	1.01	0.5	100.51	0.50
4	98.72747	3.95	2	102.44	2.05
8	99.25808	7.94	4	101.90	4.08
10	100.3153	10.03	5	103.28	5.16
12	100.7065	12.08	6	100.31	6.02
16	100.3838	16.06	8	98.25	7.86
20	100.223	20.04	10	100.19	10.02

Table 3: Recoveries of pyridoxine hydrochloride and meclizine hydrochloride in pharmaceutical formulation by complex formation.

Concentration* (μGml^{-1})	Recovered concentration	%Recovery	Concentration** (μGml^{-1})	Recovered concentration	%Recovery meclizine
20	19.96	99.81	0.5	0.49	101.31
50	50.10	100.21	2	2.08	103.18
60	59.17	98.62	4	4.01	102.66
100	97.56	97.56	5	5.16	104.05
150	151.95	101.30	6	6.18	101.08
200	193.90	96.95	8	7.91	99.00
250	245.52	98.21	10	10.11	100.95

*Pyridoxine hydrochloride, Intercept (a)=0.00004; slope (b)=0.004, Correlation coefficient= 0.9995;
**Meclizine hydrochloride, Intercept (a) = 0.0018; slope (b) = 0.0357, Correlation coefficient = 0.9997;

simultaneous equation method. All the experiments were replicated thrice. Statistical data showed that the methods were linear with correlation coefficient 0.9996 and 0.9997 for pyridoxine hydrochloride and 0.9999 and 0.9999 for meclezine hydrochloride. In complex formation method, it was linear in the range of 20 to 250 μGmL^{-1} (tables 1-3).

Precision and accuracy

To check the precision as well as accuracy of the proposed methods, independent repeatability studies were performed with five repetitions for each method. The results are summarized in tables 4-6. The methods were reproducible as well as accurate. Results of recovery studies for both methods conducted by spiking the powdered tablets with appropriate amounts of stock solution show high recovery and low standard deviation revealing the suitability of the proposed method for the determination of pyridoxine hydrochloride and meclezine hydrochloride in pharmaceutical formulations. Table 3 summarizes the statistical results evaluated from the above observations. For the accuracy of the method,

Table 4: Evaluation of precision and accuracy of pyridoxine and meclezine for simultaneous equation method

Amount of drug added (mg)	Individual amounts found (mg) mean [a]	(CV)	Amount of drug added (mg)	Individual amounts found (mg) mean [a]	(CV)
35.30	35.17	0.435	10.23	10.14	0.826
40.00	40.13	0.342	15.07	15.19	0.806
45.10	45.15	0.432	20.05	19.93	0.384
50.00	50.99	0.858	25.13	25.14	0.412
55.20	55.01	0.152	30.23	30.11	0.346
60.10	60.00	0.215	35.10	35.21	0.252

Table 5: Evaluation of precision and accuracy of pyridoxine and meclezine for simple absorbance method.

Amount of drug added (mg)	Individual amounts found (mg) mean[a]	(CV)	Amount of drug added (mg)	Individual amounts found (mg) mean[a]	(CV)
35.20	35.13	0.455	10.20	10.16	0.088
40.20	40.16	0.329	15.15	15.20	0.096
45.10	45.15	0.478	20.15	19.05	0.149
50.11	50.23	0.822	25.10	25.03	0.112
55.03	55.09	0.131	30.13	30.21	0.097
60.21	60.14	0.357	35.05	35.08	0.144

Table 6: Evaluation of precision and accuracy of pyridoxine by complexiation with ferric chloride.

Amount of drug added (mg)	Individual amounts found (mg)	Coefficient of variation (CV)	Standard error	Confidence limits [a]
20.15	19.99	0.248	0.0288	20.025 – 20.274
45.10	45.03	0.199	0.0519	44.876 – 45.323
50.19	50.10	0.094	0.0272	50.055 – 50.290
55.05	53.43	0.130	0.0416	54.950 – 55.300
60.11	59.88	0.092	0.0321	59.911 – 60.188

[a] Confidence limits at P=0.95 and two degrees of freedom.

known amount of bulk drug was spiked with known concentration of excipients. It can be seen that there was no significant influence of excipients likewise table 6 showed that, complex formation method is reliable for the determination of pyridoxine hydrochloride from tablets formulations.

Limit of detection and quantification

Detection limit (LOD) is the lowest concentration of an analyte that an analytical process can reliably detect, while limit of quantification (LOQ) is the lowest concentration of an analyte that can be measured. Under the optimum experimental conditions, the values of slopes of regression equations of proposed methods indicate good sensitivity. The small values of the variance speak about the negligible scattering of the calibration data points around the line of regressions. The high values of correlation coefficients obtained for regression equations exhibit good linearity of the methods (table 7).

CONCLUSION

Meclezine hydrochloride and pyridoxine hydrochloride can be estimated by using any of the three proposed methods. These methods have the advantages of simplicity, sensitivity, reproducibility and accuracy with high degree of precision. Since neither excipients nor meclezine hydrochloride interfere in the estimation of pyridoxine hydrochloride in tablets, the method can be used for routine quality control of meclezine

hydrochloride and pyridoxine hydrochloride in pharmaceutical formulations of all potencies and in forensic sciences involving the estimation of these drugs.

The developed methods are comparable to any HPLC method reported in the literature due their low cost and being quick and time saving. Any of these methods may be adopted as an alternative to the existing, time-consuming methods.

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