



Development and validation of spectrophotometric, HPTLC and HPLC methods for the determination of mebeverine hydrochloride and chlordiazepoxide in pharmaceutical dosage forms

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ABSTRACT

Three reliable, rapid and selective methods have been developed and validated for the determination of mebeverine hydrochloride and chlordiazepoxide in pharmaceutical dosage forms. The first method is first order derivative spectrophotometric method All variables affecting the reaction have been investigated and the conditions were optimized. The second method is based on separation of the cited drugs (mebeverine hydrochloride $R_f = 0.72 \pm 0.02$ and chlordiazepoxide $R_f = 0.43 \pm 0.04$) followed by densitometric measurement of the intact drug spots at 220 nm by HPTLC. The separation was carried on silica gel plates using chloroform: methanol: ammonia (9.5: 0.5: 0.1, (v/v/v) as a mobile phase. The linearity range was 200-1200 ng/spot for mebeverine hydrochloride and 100-600 for chlordiazepoxide. The third method is accurate and sensitive HPLC method based on separation of mebeverine hydrochloride and chlordiazepoxide on a reversed phase C18 column, using a mobile phase of potassium dihydrogen phosphate buffer (0.05 M, pH 4.0 adjusted with 0.5% orthophosphoric acid)-methanol- water (30:50:20, v/v/v) and UV detection at 260 nm in an overall analysis time of about 5 min., based on peak area. The accuracy and precision of the methods were confirmed when the standard addition technique was applied. The validation was performed according to ICH guidelines. The results obtained by applying the proposed methods were statistically analyzed.

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Introduction

Mebeverine hydrochloride (MEB) is official in IP^[1] and BP^[2]. MEB is chemically, 4-(ethyl [1-(4-methoxyphenyl) propan-2-yl] amino) butyl 3, 4-dimethoxybenzoate hydrochloride. MEB belongs to a group of compounds called musculotropic antispasmodics^[3]. These compounds act directly on the gut muscles at the cellular level to relax them. MEB is also an inhibitor of calcium-depot replenishment. Several analytical methods have been used for the determination of MEB including Spectrophotometry^[4-5], High performance liquid chromatography (HPLC)^[6-7], High performance thin layer chromatography (HPTLC)^[8]. Chlordiazepoxide (CLR) is chemically, 7-chloro-N-methyl-5-phenyl-3H-1, 4-benzodiazepin-2-amine 4-oxide^[1]. It is an anxiolytic agent and also a poor anticonvulsant^[4]. CLR is official in IP, BP and USP. The IP^[1], BP^[2] and USP^[9] describe non-aqueous titration, potentiometry titration and HPLC methods, respectively for estimation of chlordiazepoxide. Literature survey revealed determination of major impurity of CLR by UV method^[10] and HPLC method with FTIR and UV detection^[11] in formulations. Spectrophotometric^[12], Difference Spectrophotometric^[13], Micellar Liquid Chromatography^[14], derivative spectrophotometry^[15] methods were also reported for CLR with other drugs in pharmaceutical formulations^[16]. The binary mixture of the two drugs used in the treatment of gastrointestinal and colic spasm especially in the presence of psychasomatic manifestation of nervous tension, mental stress or anxiety.

Literature survey revealed only spectrophotometric methods are available for simultaneous determination of the two drugs in pharmaceutical dosage form. A successful attempt has been made to estimate these two drugs simultaneously by spectrophotometrically, HPTLC and HPLC.

Materials and Methods

A double beam UV-visible Spectrophotometer (Shimadzu, UV-1700, Japan), Camag HPTLC system (Switzerland) comprising of Camag Linomat V semiautomatic sample applicator, Camag TLC scanner 3, Camag twin trough chamber (10x10 cm), Camag Cats 4 software, Hamilton syringe (100 μ l) were used during the study. TLC plates (10x10 cm) aluminium plates precoated with 0.25 mm silica gel F254, were purchased from E. Merck. A gradient high-pressure liquid chromatograph (Shimadzu HPLC Class VP series) with two LC-10AT VP pumps, variable wavelength programmable UV/Vis detector SPD-10AVP and C18 Torrance, CA (5 micron 25 cmx4.6 mm) column from Phenomenex. was used for separation and quantification.

Mebeverine hydrochloride and chlordiazepoxide working standard, was supplied by Sun Pharmaceutical Pvt Ltds., Vadodara (India). All chemicals are of spectroscopic and chromatographic grade purchase from Merck, India Limited, Mumbai was used in the study. The commercially available marketed tablet containing a combination of mebeverine hydrochloride 135 mg and chlordiazepoxide 5 mg were procured from local pharmacy.

MEB and CLR (100 μ g/ml) were prepared in methanol for spectrophotometric, HPTLC and HPLC methods. The standard solution of both the drugs were subsequently used to prepare working standard solution for spectrophotometric, HPTLC and HPLC methods in methanol. All solutions were kept in a refrigerator at 4 °C and were stable for one week.

Twenty tablets were accurately weighed and finely powdered. An amount equivalent to 135 mg MEB and 5 mg CLR were transferred into 100 ml volumetric flask, and dissolved in 50 ml methanol. The solution was stirred with magnetic stirrer for 10 min, filtered and the volume was completed to the mark.

For HPTLC method, the plates were developed in chloroform: methanol: ammonia in the proportion of 9.5: 0.5: 0.1, (v/v/v) as a mobile phase. For detection and quantification, each of the sample solutions and standard solutions of different concentrations within the linearity range were applied as separate compact spots 12 mm apart and 15 mm from the bottom of the HPTLC plate using Linomat V applicator. The chromatographic tank was saturated with the mobile phase for 20 min before development of the plates. The plates were developed up to 8 cm in the usual ascending way, air-dried, and scanned for MEB and CLR at 220 nm by using the instrumental parameters mentioned above. For HPLC method, the mobile phase was methanol. It was filtered by using a 0.45 μ m membrane filter and degassed in an ultrasonic bath before use. The samples were also filtered by using 0.45 μ m membrane filters. The flow rate was set at 1.0 ml/min and UV detector at 260 nm. All determinations were performed at ambient temperature 27 \pm 2 °C and the injection volume was 10 μ l.

Aliquots of standard solution (100 mgml⁻¹) equivalent to (5-30 μ g ml⁻¹) for MEB and (0.5-16 μ g ml⁻¹) for CLR were transferred into 10 ml volumetric flasks separately. The volume was completed to the mark with methanol. Absorbance of the aliquots was measured at 247.20 nm for MEB and 261.0 nm for CLR. The calibration curve was plotted and the regression equation was recorded.

Aliquots of standard solution (100 mgml⁻¹) equivalent to (200-1200 ng/spot of MEB and 100-600 ng/spot of CLR) was applied to HPTLC plate by Linomat V applicator with the help of micro syringe and developed as described under chromatographic conditions previously mentioned under 'chromatographic conditions.' The plates were visualized at 220 nm and scanned at 254 nm by densitometer. Calibration curve was plotted representing the relationship between the average peak area and concentration and the regression equation was recorded.

Aliquots of standard solution (100 μ gml⁻¹) equivalent to (2-12 μ gml⁻¹) of MEB and (0.4-2.4 μ gml⁻¹) of CLR were transferred into 10-ml volumetric flasks and the volume was completed to the mark with methanol. Triplicate 10 μ l injections were made of each concentration. The average peak areas were calculated and plotted versus concentrations, linear relationship was obtained and the regression equation was recorded.

Powder from the mixed contents of 20 tablets, equivalent to 135 mg MEB and 5 mg CLR, was transferred accurately to a 100 ml volumetric flask and diluted to volume with methanol. The solution was diluted to the same concentrations of working standard solutions and treated according to the linearity for the first derivative spectrophotometry, RP-HPLC and HPTLC methods.

Results and Discussion

The first derivative spectrophotometric method is used to eliminate the spectral interference from one of the two drugs

while estimating the other drug by selecting the zero crossing point on the derivative spectra of each drug as the selected wavelength. (Fig.1) shows overlain first derivative spectra of MEB and CLR. MEB can be assayed in the presence of CLR by measuring absorption at zero crossing point of CLR in the range of 5-30 μ g/ml. The linear regression Eqn was found to be: $Y=0.008x - 0.014$, $r= 0.9995$, where Y is the absorbance value at 247.20 nm, X is the concentration in μ g/ml, and r is the correlation coefficient. CLR can be assayed in the presence of MEB by measuring absorption at zero crossing point of MEB in the range of 0.5-16 μ g/ml. The linear regression Eqn was found to be: $Y=0.015X+0.057$, $r= 0.9979$, where Y is the absorbance value at 261.0 nm, X is the concentration in μ g/ml, and r is the correlation coefficient.

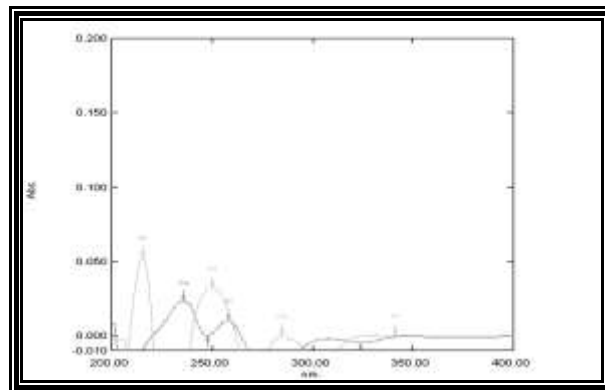


Figure 1: First derivative absorption spectra of MEB and CLR

To optimize the HPTLC parameters, several mobile phase compositions were tried. chloroform: methanol: ammonia (9.5: 0.5: 0.1, (v/v/v)), gave a sharp and symmetrical peaks of MEB and CLR with R_f values of 0.72 ± 0.02 and 0.43 ± 0.04 respectively (figure 2). Well-defined spots (and peaks) were obtained when the chamber was saturated with mobile phase vapour for 20 min at room temperature (25° C). A linear relation was obtained between peak area and the concentration of the two drugs in the range of 200-1200 ng/spot and 100-600 ng/spot for MEB and CLR, respectively. The linear regression Eqns were computed as: $Y=8.0515 X + 37.333$, $r= 0.9996$ and $Y=13.771 X + 88.616$, $r= 0.9952$, where Y is the area under the peak, X is the concentration in μ g/ml.

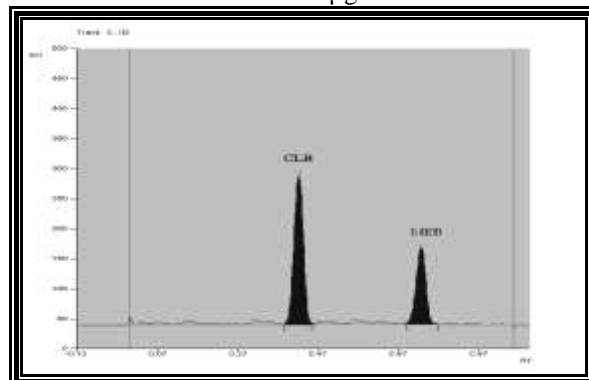


Figure 2: HPTLC Chromatogram of MEB and CLR at 220 nm

To optimize the proposed RP-HPLC method, different systems were tried for chromatographic separation of the two components by combining homogenous design and solvent polarity optimization. The best resolution was achieved using a mobile phase consisting of potassium dihydrogen phosphate buffer (0.05 M, pH 4.0 adjusted with 0.5% orthophosphoric acid)-methanol- water (30:50:20, v/v/v), which even gave good sensitivity for both drugs (figure. 3).

Table 1: System suitability test parameters for MEB & CLR for proposed HPLC and HPTLC methods

Parameters	Proposed methods			
	HPLC		HPTLC	
	MEB± % RSD ^a	CLR± % RSD ^a	MEB± % RSD ^a	CLR± % RSD ^a
Retention time, min	3.524±0.15	4.679±0.17	-	-
Rf value	-	-	0.72 ± 0.02	0.43 ± 0.04
Tailing factor	1.04±0.15	1.02±0.21	-	-
Asymmetry factor	1.14±0.25	1.18±0.29	-	-
Theoretical plates	8563.1±1.13	9563.1±1.02	-	-
Repeatability of measurement(n ^b =6)	0.62	0.32	0.56	1.21

^a RSD is a Relative standard deviation,^b n is number of determinations**Table 2: Assay results for tablets using the proposed methods**

Formulation	Proposed methods	Mix.	Label Claim (mg)		Amount of drug found (mg)		% Amount found (n ^a =3) ± SD ^b	
			MEB	CLR	MEB	CLR	MEB	CLR
Tablets	First derivative	1	135	5	134.81	4.99	99.86±0.28	99.96±0.26
	HPLC	1	135	5	135.04	5.02	100.02±0.086	100.53±1.56
	HPTLC	1	135	5	134.29	5.10	99.47±0.25	102.0±0.38

^a n is number of determinations,^b SD is a Standard deviation**Table 3: Application of the standard addition technique to the analysis of MEB and CLR in tablets by the proposed methods**

Proposed methods	Amount of drug taken (µg/ml or ng/spot)		Amount of drug added (µg/ml or ng/spot)		Amount of drug found (µg/ml or ng/spot)		% Recovery (n ^a =3) ± SD ^b	
	MEB	CLR	MEB	CLR	MEB	CLR	MEB	CLR
First derivative UV	10	1	5	0.5	14.95	1.03	99.66±0.03	98.09±0.97
	10	1	10	1	20.01	1.98	100.05±0.028	99.00±0.29
	10	1	15	1.5	24.98	2.49	99.92±0.023	99.60±0.23
HPTLC	400	200	200	100	599.85	299.65	99.97±0.65	99.88±0.65
	400	200	400	200	789.63	400.21	98.70±1.03	100.05±1.45
	400	200	600	300	989.21	501.23	98.92±0.29	100.24±1.45
HPLC	2	0.8	1	0.4	3.012	1.18	100.4±0.51	99.12±0.65
	2	0.8	2	0.8	4.036	1.60	100.9±0.49	100.13±0.63
	2	0.8	3	1.2	4.982	1.99	99.65±0.47	99.15±0.62

^a n is number of determinations,^b SD is a Standard deviation**Table 4: Summary of validation parameters for the proposed methods**

Proposed methods	Drug	Parameters			
		LOD ^a µg/ml or ng/spot	LOQ ^b µg/ml ng/spot	Interday (n = 3) (RSD ^c , %)	Intraday (n ^d = 3) (RSD ^c , %)
First derivative UV	MEB	3.0	5.0	0.39 – 0.89	0.64 – 0.90
	CLR	0.1	0.5	0.21 – 1.19	0.41 – 1.03
HPTLC	MEB	50	200	0.52-0.82	0.62-0.97
	CLR	25	100	0.23-0.45	0.18-0.71
HPLC	MEB	0.5	2.0	0.12-0.67	0.15 – 0.72
	CLR	0.1	0.4	0.13-0.49	0.35 – 0.86

^a LOD is Limit of detection,^b LOQ is Limit of quantification,^c RSD is Relative standard deviation,^d n is number of determinations

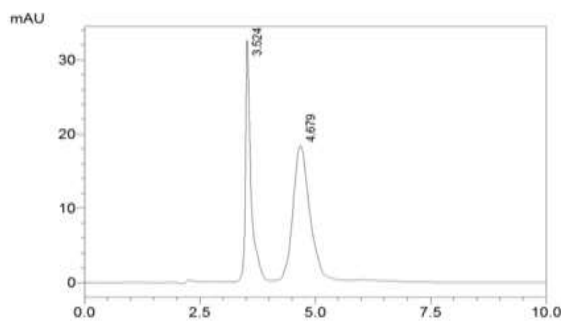


Figure 3: HPLC chromatogram of MEB and CLR at 260nm

System suitability testing of the RP-HPLC method gave good relative retention time = 1.83; theoretical plates = 8563.1 and 9536.1; asymmetry factor (A) = 1.14 and 1.18; tailing factor (T) = 1.04 and 1.02 for MEB and CLR, respectively (Table 1). A linear relation was obtained between peak area and the concentration of the two drugs in the range of 2-12 and 0.4-2.4 µg/ml for MEB and CLR, respectively. The linear regression Eqns were computed as: $Y=2516 X + 4796$, $r= 0.9990$ and $Y=10033 X + 388.1$, $r= 0.9998$, where Y is the area under the peak, X is the concentration in µg/ml, and r is the correlation coefficient. The proposed methods have been applied to assay MEB and CLR in tablets without any interference from the additives (Table 2). The validity of the suggested procedures was further assessed by applying the standard addition techniques (Table 3). The results of assay validation of the proposed methods show that they are accurate and precise according to the RSD values of intra and interday determinations (Table 4).

The methods were compared by applying the analysis of variance (ANOVA) test. The calculated F-value of 2.23 for MEB and 1.45 for CLR are less than the tabulated F-value (9.55) at the 95% confidence interval, which reveals that there is no significant difference with respect to accuracy and precision between the proposed methods. The proposed procedures can be applied for the simultaneous determination of MEB and CLR. Moreover, the methods are rapid, sensitive, accurate, precise and can be used in routine analysis.

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