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RP-HPLC METHOD DEVELOPMENT AND SIMULTANEOUS ESTIMATION OF METHYLCLOTHIAZIDE AND DESERPIDINE

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ABSTRACT

A simple reversed-phase high-performance liquid chromatographic (RP-HPLC) method has been developed and validated for simultaneous determination of methylclothiazide (MTZ) and deserpidine (DSP) in tablets. Where the RP-HPLC method was carried out on symmetry C18 (4.6 × 150mm, 5µm, make: waters) with a mobile phase containing buffer: aetonitrile (30:70), pH was adjusted to 3.5 with orthophosphoric acid (OPA) at 254nm, by an isocratic elution mode with 1ml/min flow rate using photo diode array (PDA) detector at ambient temperature. The injection volume and run time was found 20 µl and 10 minutes respectively. The retention time of MTZ and DSP was found to be 2.162 and 3.305 min. respectively. The method produced linear responses in the concentration range of 12-60 µg/ml for DSP and 20-100 µg/ml for MTZ respectively, with a correlation coefficient of 0.999 for both compounds. The limit of detection (LOD) and limit of quantification (LOQ) values for HPLC method were found to be 0.2 µg/ml and 0.5 µg/ml for methylclothiazide and 1.0 µg/ml and 1.2 µg/ml for deserpidine respectively. The recovery of the method was 98% of the labelled value. The developed method was validated according to ICH guidelines Q2 (R1) linearity was in the range of 20-100µg/ml for MTZ and 12-60µg/ml for DSP respectively his method can easily and conveniently take up for routine quantitative analysis methylclothiazide and deserpidine of bulk and pharmaceutical dosage form.

KEY WORDS

Methylclothiazide, deserpidine, method development and validation

INTRODUCTION

Analytical chemistry is an applied throughout industry, medicine and al the sciences. A drug is a substance which may have medicinal, intoxicating, performance enhancing or other effects when taken or put into a human body or the body of another animal and is not considered a food or exclusively a food (Kealey and Haines, 2002). Pharmaceutical analysis plays a very vital role in the quality assurance and quality control of bulk drugs and their formulations (Braithwaite and Smith, 1999)

Deserpidine (DSP) (figure 1), methyl (1R,15S,17R,18R,19S,20S)-18-methoxy-17-(3,4,5-trimethoxybenzoyloxy)-3,13-diazapentacyclo [11.8.0.$0^{2,10}$.$0^{4,9}$.$0^{15,20}$] henicosa-2(10),4,6,8-tetraene-19-carboxylate, is an ester alkaloid drug isolated from rauwolfia (Reeta *et al.,* 2013,) with antipsychotic and antihypertensive properties that has been used for the control of high blood pressure and for the relief of psychotic behaviour (Prabhat *et al.,* 2009).

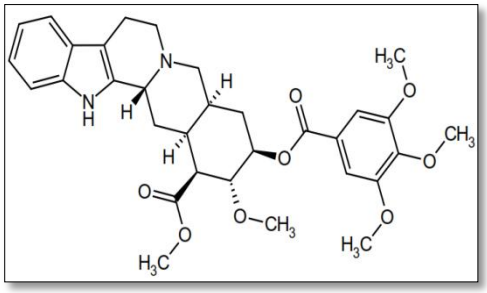

Figure 1: Chemical structures of the deserpidine

Methyclothiazide (MTZ) (Figure 2), 6-Chloro-3-(chloromethyl)-2-methyl-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine -7-sulfonamide 1,1-dioxide, is a thiazide diuretic with properties similar to those of hydrochlorothiazide.

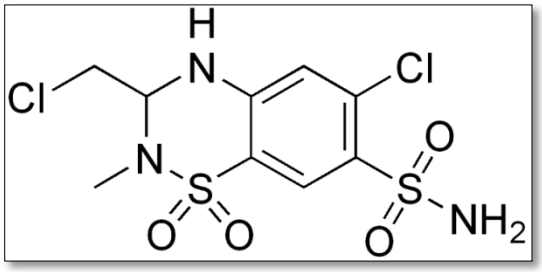

Figure 2: Chemical structure of the Methylclothiazide

MTZ was a diuretic-antihypertensive agent (Colas *et al.,* 2001), was a member of the benzothiadiazine (thiazide) class of drugs (Colas *et al.,* 2000). At maximal therapeutic dosages, all thiazides are approximately equal in their diuretic/natriuretic effects (Stepani *et al.,* 2000). The antihypertensive mechanism of MTZ is less well understood although it may be mediated through its action on carbonic anhydrases in the smooth muscle or through its action on the large-conductance calcium-activated potassium (K-Ca) channel, also found in the smooth muscle (Colas *et al.,* 2000).

There was no method reported in literature for quantitative determination of DSP and MTZ. A survey of literature (Andrea and Phyllis 1997, Yuri and Rosario 2007, Veronika 2004, Lloyd *et al.,* 1997) reveals that HPLC (in plasma) and spectrophotometric methods have been not reported for the estimation of DSP and MTZ (Craig *et al.,* 1981)

There was no stability indicating method available. In this regard, we felt it necessary to develop and validate a new rapid, economic and stability indicating RP-HPLC method for estimating deserpidine and methylclothiazide in bulk and tablet dosage forms.

MATERIALS AND METHODS

Materials and reagents

Deserpidine was kindly provided as a gift sample by Globalchem Asia Pacific, India and methylclothiazide was kindly provided from Afine Chemicals Limited, China. Other reagents such as tri ethyl amine (TEA), acetonitrile (ACN), methanol and orthophosphoric acid [HPLC grade] were purchased from the Merck [INDIA]. All other reagents used for the analysis were analytical grade. Distilled water was used throughout the investigation.

Instrumentation

The HPLC system consisted of waters alliance (Waters Corporation, MA and USA) equipped with a waters 2695 solvent delivery module in a quaternary gradient mode and waters 2669 PDA detector. Data acquisition was performed by the Empower 2 software. Analysis was carried out at 254 nm with reversed phase ODS Inertsil (4.6 × 150 mm, 5µm, make: waters) column using pH 3.5 phosphate buffer (tri ethyl amine): acetonitrile: in 30:70 ratios as the mobile phase by an isocratic elution mode with flow rate at 1ml/min. The mobile phase was degassed and filtered through 0.45 µm membrane filter before pumping into HPLC system.

Preparation of Solutions

Preparation of Buffer Solution (pH 3.5)

Weighed 7 gm of potassium di hydrogen ortho phosphate into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water. Adjust 3.5 pH with orthophosphoric acid.

Preparation of mobile phase

Mix a mixture of above buffer 250ml (25%), 750ml of acetonitrile HPLC (75%) and degas in ultrasonic water bath for 5 min. filter through 0.45µm filter under vacuum filtration.

Diluent preparation: Mobile phase used as diluent.

Selection of flow rate

A chromatogram was run with the optimized mobile phase and some different flow rate of 0.8 ml/min, 1.0 ml/min. and 1.2 ml/min. were tried. The best retention time and separation was obtained at 1.0 ml/min. so, the flow rate of 1.0 ml/min. has been selected.

Preparation of standard solution

Accurately weigh and transfer 12 mg of DSP and 10mg of MTZ working standard into a 10ml clean and dry volumetric flask and about 7ml of diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent (stock solution). Further pipette 0.3 and 0.6 ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluents.

Selection of analytical wavelength

By appropriate dilutions of the standard stock solutions with menthol, various concentration of DSP and MTZ were prepared separately and their overlain spectra was obtained using the double beam UV visible spectrophotometer in the spectrum mode between the wavelength ranges of 200 nm to 400 nm. From the overlain spectra, it was observed that deserpidine and methylclothaizide exhibited strong absorbance at about 254 nm, which was selected as the analytical wavelength for further analysis. (Figure 3)

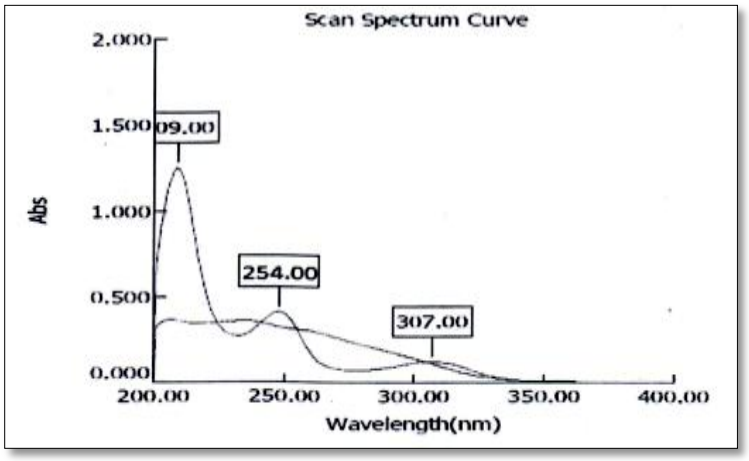

Figure 3: Scan spectrum curve of deserpidine and Methylclothiazide

Chromatographic parameters

Equipment	:	HPLC equipped with Auto Sampler
Column	:	C18 ODS Inertsil (4.6 × 150mm, 5µm, make: waters).
Flow rate	:	1ml/min.
Detector	:	PDA
Wavelength	:	254 nm
Injection volume	:	20 µl
Column oven	:	Ambient

Run time : 10 min.

METHOD DEVELOPMENT

Many trials have been performed by different mobile phases with various concentrations. After observing the theoretical plates stability factor, both peaks are eluted clearly more plate count and more resolution was observed to compare with other trials. Then the above chromatography parameters were chosen.

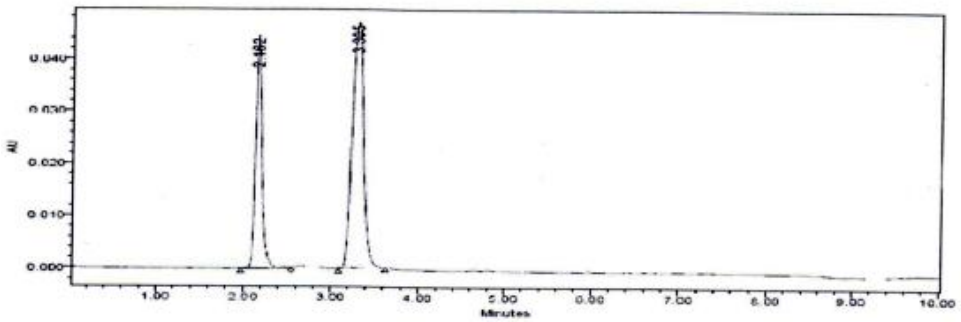

Figure 4: Chromatogram with pH 3.5 phosphate buffer:Acetonitile = 30:70 of mobile phase

System suitability

System suitability studies were carried out as specified in the United States of Pharmacopoeia (USP). These parameters include column efficiency, resolution, capacity factor, tailing factor and HETP were calculated in present study and it shown in table 1 and table 2.

Table 1: System suitability study parameters for methylclothaizide

Sl. No.	System suitability parameters	MTZ
1	Resolution	**6**
2	Tailing factor	1.2
3	No. of theoretical plate	3724
4	Retention time	3.3

Table 2: System suitability study parameters for deserpidine

Sl. No.	System suitability parameters	DSP
1	Resolution	6.2
2	Tailing factor	1.2
3	No. of theoretical plate	4650
4	Retention time	2.162

Specificity

The prepared blank (diluent) has been injected into HPLC as per methodology. Blank chromatogram should not show any peaks at the retention times of the analyte peaks.

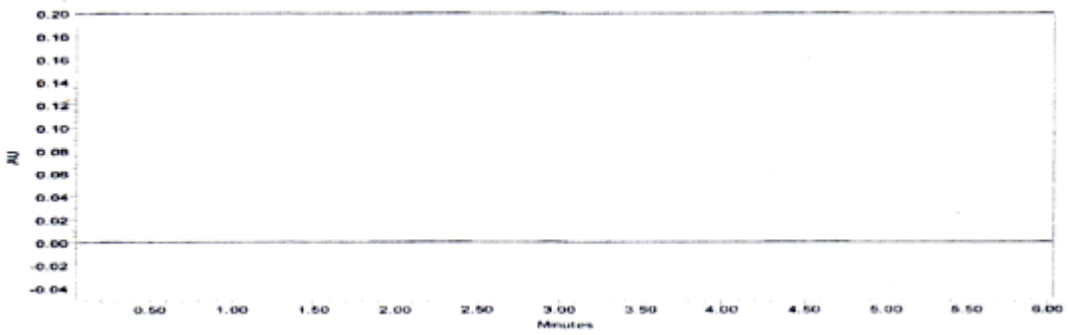

Figure 5: Blank chromatogram

Linearity

Inject each level into the chromatographic system and measure the peak area. Volume of 20 µl of sample was injected for each concentration level and calibration curve was constructed by plotting the peak area versus the drug concentration (on X-axis concentration and on Y-axis peak area) and calculate the correlation coefficient.

Table 3: Linearity results of deserpidine

Sl. No.	Linearity level	DSP		MTZ	
		Concentration (ppm)	Area	Concentration (ppm)	Area
1	I	12	364052	20	544062
2	II	24	672802	40	1072605
3	III	36	1022619	60	1535308
4	IV	48	1374938	80	2040501
5	V	60	1728316	100	2625141

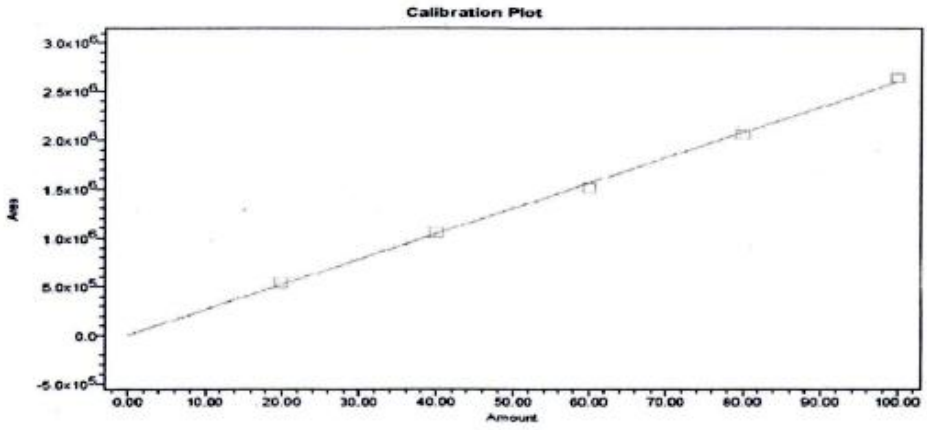

Figure 6: Methylclothiazide calibration curve

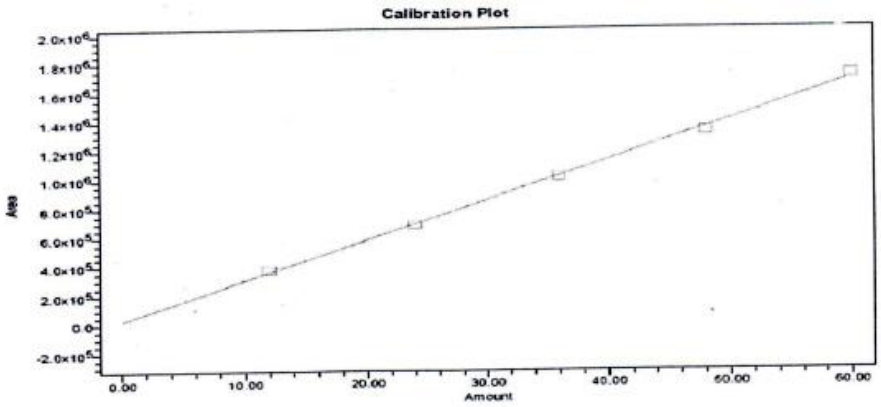

Figure 7: Deserpidine calibration curve

Precision

The standard solution was injected for six times and measured the area for all six injections in HPLC. The % RSD for the area of six replicate injections was found to be within the specified limits.

Table 4: Precision results of deserpidine.

Sl. No.	Injection	System precision	Intermediate precision
		Area	Area
1	Injection-1	1020585	1024722
2	Injection-2	1051075	1015087
3	Injection-3	1021924	1017135
4	Injection-4	1054299	1016549
5	Injection-5	1072159	1038435
6	Injection-6	1054008	1023265
7	Average	1011008.4	1020255.5
8	Standard deviation	4674.6	6867.6
9	% RSD	0.4	0.9

Table 5: Precision results of methylclothiazide

Sl. No.	Injection	System precision	Intermediate precision
		Area	Area
1	Injection-1	1523391	1496209
2	Injection-2	1523391	1507963
3	Injection-3	1516673	1521163
4	Injection-4	1550819	1522810
5	Injection-5	1560819	1528916
6	Injection-6	1525018	1515412
7	Average	1515018.7	1515412.0
8	Standard deviation	24108.8	13175.7
9	% RSD	1.4	0.9

Accuracy

Accuracy was performed in triplicate for various concentrations of Sample solutions, prepared by spiking at about 50%, 100% and 150% of specification limit to Placebo and analyzed by the proposed HPLC method and calculate the amount found and amount added for DSP and MTZ and calculate the individual

recovery and mean recover values shown in table 6 and table 7.

Table 6: Accuracy results of methylclothiazide

Sl. No.	% Concentration (at specific level)	Area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
1	50 %	774787.7	5	5.0	101.3%	
2	100 %	1537580	10	10.0	100.3%	100.3%
3	150 %	2285575	15	14.9	99.4%	

Table 7: Accuracy results of deserpidine

Sl. No.	% Concentration (at specific level)	Area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
1	50 %	605652.5	6	5.8	98.1%	
2	100 %	1246314	12	12.1	101.0%	100.0%
3	150 %	1869868	18	18.1	101.0%	

Robustness

The robustness was determined by analysis of aliquots from homogenous lots by differing physical parameters like flow rate (table 8) and mobile phase composition (table 9) which may differ but the responses were still within the specified limits of the assay.

Table 8: Robustness results

Drug name	Flow rate (ml/min.)	USP plate count	USP tailing
	0.8	4469.0	1.3
Deserpidine	1.0	4740.0	1.2
	1.2	4089.0	1.2
	0.8	3076.0	1.1
Methylclothiazide	1.0	3734.0	1.2
	1.2	30612.0	1.1

Standard solution 36 µg/ml of deserpidine and 60 µg/ml of methylclothaizide was prepared and analysed using the varied mobile phase composition along with the actual moble phase composition in the method.

Table 9: Robustness results

Drug name	Variation in mobile phase composition	USP plate count	USP tailing
	0.8	2018.0	0.9
Deserpidine	1.0	4740.0	1.2
	1.2	2012.0	1.0
	0.8	3025.0	1.0
Methylclothiazide	1.0	3714.0	1.2
	1.2	3012.0	1.0

Limit of Detection (LOD) and Limit of Quantification (LOQ)

Calibration curve was repeated for 3 times and the standard deviation (SD) of the intercepts was calculated. The LOD and LOQ were determined by the following formulas:

LOD = 3.3 σ/S

LOQ = 10 σ/S

Where σ = Standard deviation of Intercepts of calibration curves; S = Mean of slopes of the calibration curves.

The slope 'S' may be estimated from the calibration curve of the analyte.

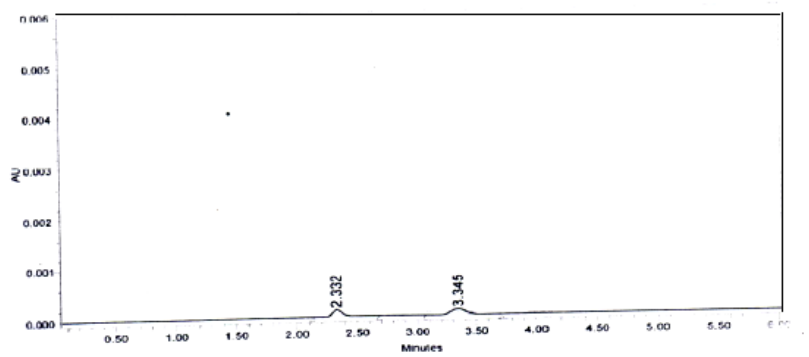

Figure 8: LOQ of deserpidine and methylclothiazide

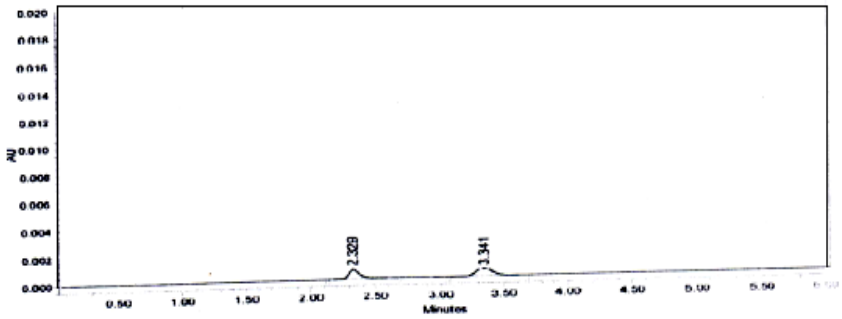

Figure 9: LDQ of deserpidine and methylclothiazide

RESULTS AND DISCUSSION

The suitability of the system was studied by the values obtained for theoretical pate, resolution and tailing factor of the chromatogram of standard drugs and presented. The selectivity of the method was revealed by the repeated injection of mobile phase and no interference was found (table 1 and table 2). The standard drug solution was varying in concentration ranging from 12-60 µg/ml for DSP and 20-100 µg/ml for MTZ. The co-efficient correlation was found to be 0.999 for both drugs. The calibration graph shows that liner responses were obtained over the range of concentration used in the assay procedure.

Several proportions of buffer and solvents were evaluated in order to obtain suitable composition of the mobile phase. Various experiments were performed by changing the concentration and pH of mobile phase, stationary phase selection etc., to optimize the chromatographic conditions to achieve better efficiency of the chromatographic system. The composition of mobile phase buffer: acetonitrile (30:70) of pH 3.5 with flow rate of 1ml/min. and runtime of 10 min. at 254nm perfect chromatogram was eluted. Peaks were eluted properly and retention time of peak is less than 10 min. Linearity results were shown in table 3.

The precision of the method was demonstrated by system and method precision. All solutions were injected into the chromatographic system. The peak area and percentage relative standard deviation were calculate and presented in tables (table 4 and table

5). And the % RSD for the area of five standard injections results should not be more than 2%.

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out by preparing 3 individual samples with same procedure from the formulation and injecting. From the data obtained added of standard drugs were found to be accurate. The average % recovery of MTZ and DSP were calculated. The accuracy results were given in table 6 and table 7. The mean % recovery at each spike level should be not less than 98.0% and not more than102.0 %.

The robustness of the method was studies by carrying out experiments by changing conditions discussed earlier. The response factors for this changed chromatographic parameter were almost same as that of the fixed chromatographic parameters (table 8 and table 9) and hence developed method is said to be robust and ruggedness.

The limit of detection (LOD) of MTZ and DSP were determined according to the ICH guidelines (International Conference on Harmonization 1995, 1996 and 2005), and found to be 0.2µg/ml and 1.0µg/ml respectively shown in figure 8.

The limit of quantification (LOQ) of MTZ and DSP were determined according to the ICH guidelines, and found to be 0.5µg/ml and 1.2µg/ml respectively shown in figure 9.

The above motioned results was performed based on ICH guidelines, the method was validated with system suitability, linearity, accuracy, precision, LOD and LOQ. All the results were summarized in the table 10.

Table 10: Results obtained by RP-HPLC method

Sl. No.	Parameter	Methylclothiazide	Deserpidine
1	Accuracy	% Recovery = 100.0%	% Recovery = 100.3%
2	Precision	% RSD = 1.4 %	% RSD = 0.4 %
3	Id precision	% RSD = 0.9 %	% RSD = 0.9 %
4	Linearity	R^2=0.999	R^2=0.999
5	Rang	12-60 ppm	20-100 ppm
6	Limit of detection	0.2µg/ml	1.0µg/ml
7	Limit of quantification	0.5µg/ml	1.2µg/ml

CONCLUSION

The method was validated in terms of sensitivity, accuracy and precision and can be used for the routine determination of DSP and MTZ pharmaceutical formulations. The proposed method does not suffer from any interference due to common excipients.

The results indicating that the proposed method was precise, accurate, specific and simple. This method was validated statistically and the results of recovery studies were in good agreement with the respective label claim of the formulation. Thus the method is less time consuming and can be employed for routine batch analysis of MTZ and DSP. Therefore the proposed RP-HPLC method could be successfully applied to estimate commercial pharmaceutical products containing methylclothiazide and deserpidine.

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