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Liquid Chromatographic Estimation of Dosulepin HCl and Methylcobalamin in Pharmaceutical Formulation.

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Research Article

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ABSTRACT

A sensitive, selective and precise high performance liquid chromatographic method has been developed and validated for the simultaneous determination of Dosulepin and Methylcobalamin both as a bulk drug and in formulation. The method employed Luna C18 column (250 x 4.6 mm id, 5 µm particle size) as the stationary phase while Acetonitrile, 0.02 M KH2P04and Methanol(50:40:10v/v, pH 6) was used as mobile phase. The Rtof Dosulepin and Methylcobalamin were observed to be 4and 2.1 minutes, respectively. Analysis was carried out in absorbance mode at 260 nm. The linear regression analysis data for the calibration plots showed a good linear relationship for Dosulepin and Methylcobalamin over a concentration range of 0.05-20 µg/ml and 0.1-20 µg/ml respectively with correlation co-efficient of 0.999forDosulepin and 0.9991 for methylcobalamin. The LOQ was found to be 0.05 and 0.1 µg/ml respectively for Dosulepin and Methylcobalamin. The method was validated as per ICH guideline and it was found to beaccurate, precise and robust. Marketed formulation was analyzed successfully.

INTRODUCTION

DOS is chemically3-(6*H*-dibenzo [*b,e*]thiepin-11-ylidene)propyldimethylamine hydrochloride[1,2]. DOS is a Tricyclic antidepressant (TCA) which inhibit the active reuptake of biogenic amines NA and 5-HT in to their respective neurons [3]. MCA is chemically 3-[({2-[(diaminomethylidene) amino]-1, 3-thiazol-4-yl}methyl)sulfanyl]-N'-sulfamoylpropanimidamide[4]. MCA is active form of vitB12[5].Literature survey revealed there is no published chromatographic method for this combination of drug. The present paper describes a simple, accurate and precise method for reverse phase liquid chromatographic estimation of DOS and MCA in combined tablet dosage form. The proposed method is optimized and validated as per the International Conference onHarmonization (ICH) guidelines [6,7]. In the present work, a successful attempt has been made to estimate both these drugs simultaneously by RP-HPLC method [8,9,10].

MATERIALS AND METHODS

Instruments

Instrument Perkin Elmer USA, Series 200, Phenomenex Luna C18 column (250 x 4.6 mm id, 5µm particle size)was used for analytical method development. The chromatographic data were processed byTotalchrom navigator HPLC version 6.3.1Software.

Materials

Prothiaden M (75 mg DOS and 1.5 mg MCA) manufactured by Abbott India Ltd. All chemicals and reagents used were of AR grade.

Reagents: All the chemicals used were of AR grade.

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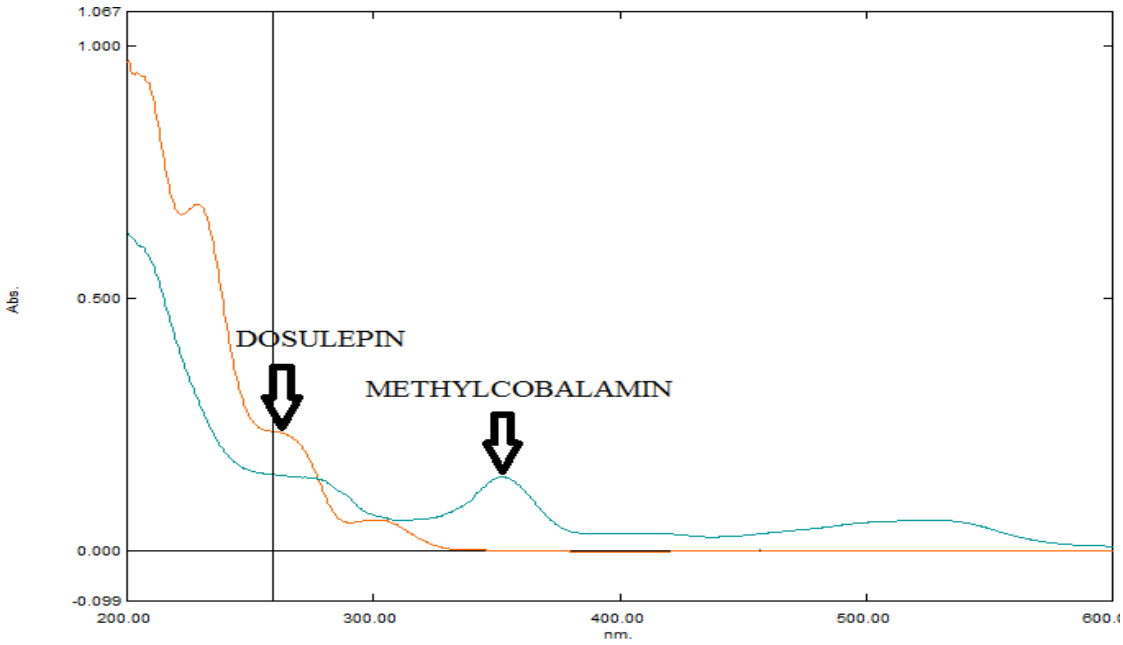
Selection of Analytical wavelength:

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is the one that gives good response for the drugs that are to be detected. Overlay UV spectra of both the drugs showed that DOS and MCA absorbed appreciably at 260 nm, so detection was carried at this wavelength. (Figure 1).

Figure 1: Overlain Zero Order Spectra of 10 ppm Solutions of Dosulepin hcl and Methylcobalamin in Water



Preparation of Mobile Phase

2.72 gm of KH_2PO_4 was weighed and dissolved in 1000 ml of HPLC water to prepare 0.02 M KH2PO4 buffer. Mobile phase was prepared by mixing 500 ml of Acetonitrile, 400 ml of 0.02 M KH2PO4 and 100 ml Methanol.The pH was adjusted to 6 using o-phosphoric acid (1%). Solution was filtered through Whatman filter paper No. 41 and sonicate for 10 min and this solution was used as a mobile phase.

Preparation of Standard Stock Solutions

DOS (10 mg) and MCA (10 mg) were accurately weighed and transferred to two separate 10 ml volumetric flask and dissolved in fewml of methanol. Volumes were made up to the mark with methanol to yield a solution containing 1000µg/ml of DOS and 1000µg/ml of MCA, respectively. Appropriate aliquots from above solution were taken and diluted with mobile phase to obtain 100 µg/ml of DOS and 100µg/ml of MCA, respectively.

Chromatographic conditions

A Phenomenex® C-18 (250 x 4.6 mm id) chromatographic column equilibrated with mobile phase Acetonitrile: 0.02 M KH2PO4: Methanol (50: 40: 10, % v/v).pH 6 was adjusted using o-phosphoric acid (1%). Mobile phase flow rate was maintained at 1 ml/min and effluents were monitored at 260 nm. The sample was injected using a 20 µL fixed loop, and the total run time was 6 min.

Calibration Curve for EPL and MCA

Appropriate aliquot of stock solution of DOS and MCA was taken in same 10 ml volumetric flasks. The volume was made up to the mark with mobile phase to obtain final concentration of 0.05, 0.1, 0.5, 1, 5, 10, 20 µg/ml of DOS and 0.1, 0.2, 0.5, 1, 5, 10, 20µg/ml of MCA, respectively.

RESULTS

Validation Parameter

Linearity and Range

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The calibration curve for DOS was found to be linear in the range of 0.05-20 µg/ml with a correlation coefficient of 0.9995. The calibration curve for MCA was found to be linear in the range of 0.1-20 µg/ml with a correlation coefficient of 0.9997. The regression analysis of calibration curves are reported in table 1- 2& figure 3-4.

Figure 2: acetonitrile: 0.02 M KH_2PO_4 Buffer: Methanol (50:40:10)

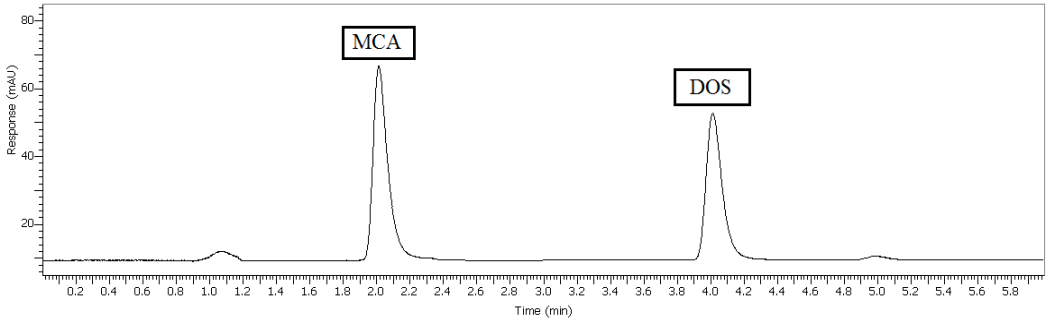


Figure 3: Calibration Curve of DOS by HPLC method

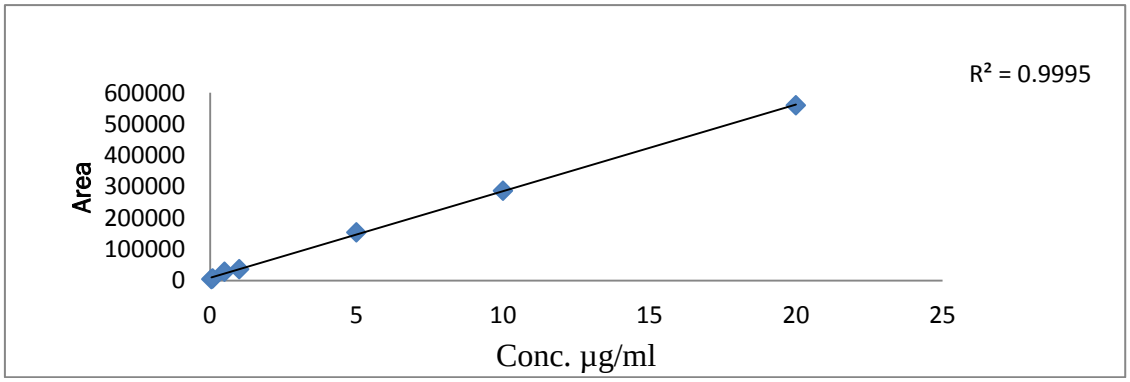
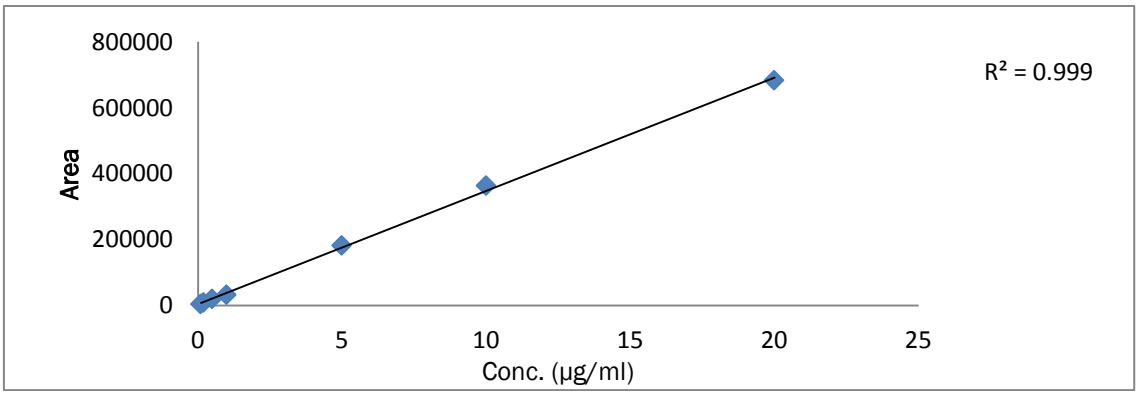


Figure 4: Calibration Curve of MCA by HPLC method



Precision

Intraday precision

The intraday studies were carried out by measuring response for 3 concentrations for 3 times a day. The % RSD values for DOS& MCA were found to be 0.39- 1.25 and 0.54- 1.00 for intradayrespectively. These %RSD value was found to be less than ± 2.0 indicated that the method is precise (Table 3 & 4).

Interday precision

The interday studies were carried out by measuring response for 3 concentrations for 3 times at 3 different days.

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The % RSD values for DOS & MCA were found to be 0.47-1.60 and 0.72- 1.25 for interday respectively.These %RSD value was found to be less than ± 2.0 indicated that the method is precise (Table 3 & 4).

Repeatability

The repeatability studies were carried out by measuring response for a single concentration for 6 times a day. The % RSD values of repeatability for DOS and MCA were found to be 0.56 and 0.63 (Table 5). These %RSD value was found to be less than ± 2.0 indicated that the method is precise.

Accuracy

The accuracy of the method was determined by calculating recoveries of DOS & MCA by method of standard addition. The recoveries found to be 99.42 – 101.89 % and 109.58– 110.47 % for DOS & MCA respectively (Table 6).

Limit of detection and limit of quantification

LOD is the lowest amount of the analyte that can be detected but not quantified. From the visual observation of chromatogram, the detection limits for DOS & MCA were 0.02 and 0.04 µg/ml, respectively. LOQ is the lowest amount of the analyte that can be detected and quantified. The quantitation limits were 0.05 and 0.1 µg/ml respectively. The above data shows that a microgram quantity of both the drugs can be accurately and precisely determined.

Robustness

Robustness study was carried out by changing the mobile phase composition, pH and flow rate (Table7). There is no significant change in the results so the proposed method is robust.

Solution stability

Stability of standard and sample solution of DOS and MCA were evaluated at room temperature for 24 hr. Both the drugs were found to be stable with a recovery of more than 98% (Table 8).

System suitability parameters

System suitability test was carried out and the results are summarized in Table 9

Analysis of marketed formulation

Marketed formulation was analyzed using proposed method which gave percentage recovery for DOS & MCA were 99.49 and 110.01respectively. Rt of 4 min and 2.1 min were observed in the chromatogram for DOS and MCA, and no interference from the excipients present in the marketed tablet formulation was observed (Table 11).

Table1: Result of calibration readings for DOS by HPLC method

Concentrations (µg/ml)	Area Mean ± S.D. (n=5)	% RSD
0.05	5083.91 ± 64.78	1.27
0.1	6761.09 ± 74.43	1.10
0.5	28120.41 ± 223.56	0.79
1	36332.15 ± 230.13	0.63
5	153780.92 ± 912.98	0.59
10	286716.97 ± 1340.12	0.46
20	559593.46 ± 2659.21	0.47
SLOPE	27644.6 ± 104.95	
INTERCEPT	9031.02 ± 199.56	
R2	0.9995	

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Table 2: Result of calibration readings for MCA by HPLC method

Concentrations (µg/ml)	Area Mean ± S.D. (n=5)	% RSD
0.1	4490.56 ± 61.61	1.37
0.2	9629.64 ± 97.31	1.01
0.5	19499.63 ± 126.31	0.64
1	32608.44 ± 245.08	0.75
5	181729.53 ± 1470.96	0.80
10	363784.37 ± 2190.47	0.60
20	682785.22 ± 5380.51	0.78
SLOPE	34375.27 ± 258.18	
INTERCEPT	4216.06 ± 545.48	
R2	0.9997	

Table 3: Precision data for DOS

Conc. µg/ml	Intraday (n=3) (area) ± S.D	% RSD	Interday (n=3) (area) ± S.D	% RSD
0.05	5041.01 ± 63.40	1.25	5046.99 ± 80.96	1.60
1	36257.86 ± 206.67	0.57	36270.93 ± 276.75	0.76
20	558192.84 ± 2185.98	0.39	559168.13 ±2680.52	0.47

Table 4: Precision data for MCA

Conc. µg/ml	Intraday (n=3) (area) ± S.D	% RSD	Interday (n=3) (area) ± S.D	% RSD
0.1	4521.20 ± 45.34	1.00	4529.96 ± 56.81	1.25
1	32637.64 ± 208.72	0.63	32633.17 ± 286.09	0.87
20	687672.73 ± 3713.63	0.54	685208.21 ± 4942.78	0.72

Table 5: Repeatability data for DOS and MCA

Concentration	DOS 1 µg/ml	DOS Rt (min)	MCA 1 µg/ml	MCA Rt (min)
Area	36527.52	4.12	32975.25	2.15
	36274.49	4.09	32772.74	2.13
	35996.76	4.11	32389.95	2.14
	36454.84	4.07	32843.38	2.11
	36549.41	4.05	32642.93	2.09
	36398.87	4.03	32589.47	2.12
Mean.	36366.98	4.0783	32702.29	2.1233
Std. Dev.	206.59	0.03	206.48	0.02
% RSD	0.56	0.85	0.63	0.71

Table 6: Accuracy data of DOS & MCA by the proposed HPLC method

Amount of drug added initially from formulation		Amount of standard drug added (µg/ml)		% recovery Mean ±SD (n = 3)		% RSD	
DOS	MCA	DOS	MCA	DOS	MCA	DOS	MCA
5	5	0	0	101.89± 0.68	110.26± 0.83	0.66	0.76
5	5	2.5	2.5	99.76± 0.58	109.58± 0.89	0.59	0.81
5	5	5	5	99.42± 0.69	110.47 ± 0.90	0.70	0.82
5	5	7.5	7.5	100.44± 0.89	110.06± 0.90	0.88	0.82

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Table 7: Robustness results of DOS & MCA in given formulations

Parameter	Method condition	Rt		% RSD of peak area	
		DOS	MCA	DOS	MCA
Mobile phase ratio	50: 35: 15	3.78	1.85	0.64	0.68
	50:45: 5	4.72	2.27	0.59	0.62
pH	5.5	3.84	1.91	0.57	0.59
	6.5	4.33	2.24	0.63	0.64

Table 8: Solution stability study

Time (Hrs.)	Area (n=3)		% Recovery	
	DOS 1 (µg/ml)	MCA 1 (µg/ml)	DOS	MCA
0	36676.05	38591.11	100	100
3	36525.63	38286.13	99.59	99.21
6	36367.92	38097.04	99.16	98.72
24	36265.23	37896.36	98.88	98.20

Table 9: System suitability

Parameters	DOS	MCA
Assymetricfactor	1.13	1.11
Resolution	2.11	
Theoretical Plates	3541.57	4472.42

Table 10: Summary of Validation Parameters of HPLC

Parameters	DOS	MCA
Range(µg/ml)	0.05- 20	0.1- 20
Retention time (min)	4	2.1
Assymetricfactor	1.13	1.11
Resolution	2.11	
Theoretical Plates	3541.57	4472.42
Intra-day Precision (n=3) (%RSD)	0.39- 1.25	0.54- 1.00
Inter-day Precision (n=3) (%RSD)	0.47-1.60	0.72- 1.25
Repeatability(%RSD)	0.56	0.63
Accuracy (%Recovery)	99.42 – 101.89 %	109.58– 110.47 %
Detection limit (µg/ml)	0.02	0.04
Quantitation limit (µg/ml)	0.05	0.1
Robustness	Robust	Robust
Specificity	Specific	Specific

Table 11: Analysis of marketed formulation

Formulation	Label Amount (mg)		Amount found (mg)		% of drug found ± SD	
	DOS	MCA	DOS	MCA	DOS	MCA
Prothiaden M	5	5	5.06	5.52	101.27± 0.67	110.51± 0.67

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CONCLUSION

The HPLC method for the estimation of DOS and MCA has been developed. The proposed method was validated as per ICH Q2 (R1) guideline for accuracy, precision, linearity, specificity and robustness. The developed method was successfully applied to marketed formulation.

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