

**INTERNATIONAL JOURNAL OF UNIVERSAL PHARMACY
AND BIO SCIENCES****IMPACT FACTOR 4.018*******ICV 6.16*******Pharmaceutical Sciences****Research Article.....!!!****“SIMULTANEOUS DETERMINATION AND VALIDATION OF BUPROPION AND
DEXTROMETHORPHAN IN BULK DRUG AND PHARMACEUTICAL FORMULATIONS”****Deepa*, Anil Kumar S M, Vijayakrishna C Aradhya**Department of pharmaceutical Analysis, National College of Pharmacy, Balraj Urs Road,
Shimoga-577201, Karnataka, India**KEYWORDS:**Bupropion, Dextromethorphan,
Simultaneous equation method,
Q-Absorbance method ,
Validation.**FOR CORRESPONDENCE:****Deepa*****ADDRESS:**Department of Pharmaceutical
Analysis, National College of
Pharmacy, Balraj Urs Road,
Shimoga-57720, Karnataka,
India.**ABSTRACT**

Background: The combination of bupropion and dextromethorphan is prescribed for treating major depressive disorder and agitation in Alzheimer’s disease via inhibition of CYP2D6.

In the present study a simple, rapid and sensitive UV spectrophotometric methods have been developed for the simultaneous estimation of Bupropion and Dextromethorphan in bulk drug and pharmaceutical formulations. **Methods:** Method A: Simultaneous equation method was based on the measurement of absorbance at two selected wavelengths 251nm and 277 nm for the estimation of Bupropion and Dextromethorphan respectively. Beer’s law obeyed in the concentration range of 5-20µg/ml and 20-100 µg/ml with ($r^2=0.9989$, and $r^2=0.9999$) for Bupropion and Dextromethorphan respectively. LOD of both drugs were 0.2660µg/ml and 1.7277 µg/ml and LOQ were found to be 0.8062 µg/ml and 5.2356µg/ml for Bupropion and Dextromethorphan respectively. Method B: Q-Absorbance method is based on the measurement of absorbance at two selected wavelengths 267nm (Iso-absorptive point) and 277nm for the estimation of Bupropion and Dextromethorphan respectively. Linearity range was found 5-25 µg/ml and 20-100 µg/ml with ($r^2=0.9291$, and $r^2=0.999$) Bupropion and Dextromethorphan respectively. In both the methods the % RSD for intra-day and inter-day precision was within 2%. **Conclusion:** Proposed techniques were effectively utilized for the simultaneous determination of Bupropion and Dextromethorphan from tablet preparation. The outcomes of the proposed procedures were compared with the earlier described methods and no statistical difference was found between the methods in terms of accuracy and precision.

INTRODUCTION:

Bupropion⁸⁻¹² (BUP) is a 2-(tetra-butyl amino)-1-(3-chloro phenyl) propan-1-one. BUP is atypical antidepressant. BUP is a norepinephrine/dopamine-reuptake inhibitor (NDRI) that exerts its pharmacological effects by weakly inhibiting the enzymes involved in the uptake of the neurotransmitters norepinephrine and dopamine from the synaptic cleft, therefore prolonging their duration of action within the neuronal synapse and the downstream effects of these neurotransmitters. More specifically, BUP binds to the norepinephrine transporter (NET) and the dopamine transporter (DAT).

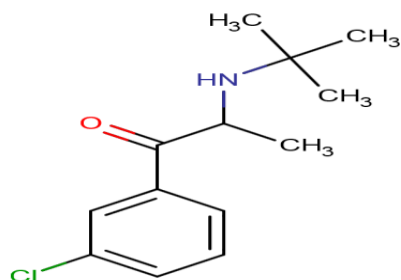


Fig.1 Structure of Bupropion

Dextromethorphan¹³⁻¹⁵ (DMF) is a (4*b*S,8*a*R,8*a*S,9*S*)-3-Methoxy-11-methyl-6,7,8,8*a*,9,10-hexhydro-5*H*-9,4*b*-(epiminoethanol). DMF exhibits antitussive activity and is devoid of analgesic or addictive property. This agent crosses the blood-brain-barrier and activates sigma opioid receptors on the cough center in the central nervous system, thereby suppressing the cough reflex.

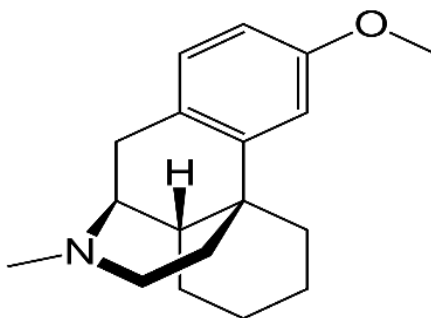


Fig.2 Structure of Dextromethorphan

The combination of BUP and DMF is prescribed for treating major depressive disorder and agitation in Alzheimer's disease via inhibition of CYP2D6.

On literature survey, since bupropion is an official drug in IP, several analytical techniques like RP-HPLC, HPTLC and spectrophotometric methods for the estimation of BUP individually and in

combination with other drugs have been reported. DMF in combination with other drugs has been estimated by HPLC and spectrophotometric methods.

Since, no method has been reported for the simultaneous estimation of BUP and DMF in combined dosage form and no method is available in the pharmacopoeia. In the view of the need for suitable method for routine analysis in combined formulations, a new simple, precise and accurate analytical methods for simultaneous estimation of tilted drugs has been developed and extended it for their determination in formulations.

MATERIALS AND METHODS:

Instrument: Spectrophotometric measurements were performed using double-beam UV-Vis spectrophotometer. (1800, Shimadzu) UV-visible Spectrophotometer with spectral band width of 1 ± 0.2 nm, wave length accuracy ± 0.3 nm and a pair of quartz cuvettes having 1 cm path length was used. Volumetric flasks used for preparation of standard solutions and sample solutions were calibrated before use.

Chemicals:

Standard analytes of BUP and DMF were purchased from Cadila health care (Ahmedabad, India) and Brawn laboratories limited (Haryana, India). Distilled water used for the dilution of standard and sample solutions.

METHODS:

Preparation of standard solutions:

Standard solutions of BUP and DMF Prepared by 100 mg powder weighed accurately and dissolved in 100ml of water respectively. Further standard solutions were diluted using water to obtain a concentration in the linearity range.

Preparation of sample solution

The tablet consisting of BUP and DMF was not available in the local market (AXS-05 containing BUP-105 mg and DMF-45 mg), hence a in-house preparation has been made consisting of BUP, DMF and all tablet excipients was prepared by geometrically adding sufficient amount BUP, DMF. Powder equal to 100 mg of BUP was accurately weighed and transferred to 100 ml volumetric flask and dissolved in water. Then further dilutions were done with water to get both the concentrations of the drugs within their range respectively (by standard addition method).

Procedure for construction of calibration curve:

Required amount of standard solutions of BUP and DMF were transferred into the 10 ml volumetric flasks to get the five solutions in the concentration range of 5-25 µg/ml of BUP and 20-100 µg/ml DMF separately. All these solutions were scanned in the range of 200 – 400 nm against water as blank and spectra were recorded., Simultaneous equation method was based on the measurement of absorbance at two selected wavelengths 251nm and 277nm for the estimation of BUP and DMF respectively. Further, Q-Absorbance method is based on the measurement of absorbance at two selected wavelengths 267 nm (Iso-absorptive point) and 277nm for the estimation of BUP and DMF respectively. The calibration curves were created for both analytes by plotting a graph between peak absorbance against corresponding concentrations. In addition, regression equations were figured.

$$C_x = \frac{A_2 a_{y1} - A_1 a_{y2}}{a_{x2} a_{y1} - a_{x1} a_{y2}}$$

$$C_y = \frac{A_1 a_{x2} - A_2 a_{x1}}{A_{x2} a_{y1} - a_{x1} a_{y2}}$$

1. Absorptivity of X at λ_1 and λ_2 , a_{x1} and a_{x2} respectively.
2. Absorptivity of Y at λ_1 and λ_2 , a_{y1} and a_{y2} respectively.
3. The absorbance of the diluted sample at λ_1 and λ_2 , A_1 and A_2 respectively

RESULTS AND DISCUSSION

Simultaneous equation method the absorbance was measured at 251 nm and 277 nm for the estimation of BUP (Fig. 3) and DMF (Fig. 4) respectively and Overlay spectra of mixture in fig.(5) and calibration plot of BUP and DMF were (Fig.7) (Fig.9) and (Fig.8)(Fig.10) respectively.

In Q-absorbance method respectively, the absorbance was measured at 267 nm and 277 nm for the estimation of BUP and DMF.(Fig.6). calibration plot of BUP and DMF were (Fig.11) and (Fig.12) respectively.

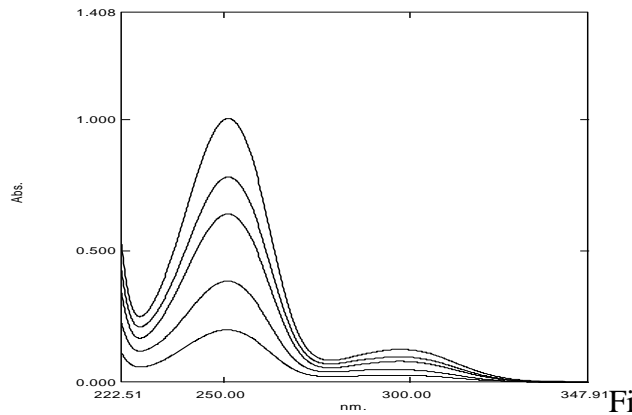


Fig.3 Overlay spectrum of BUP at 251 nm

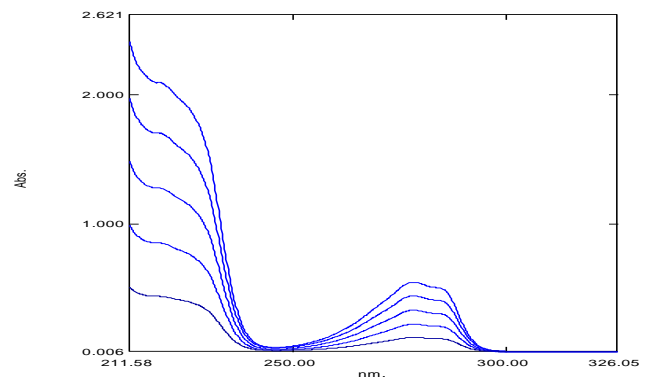


Fig.4 Overlay spectrum of DMF at 277 nm.

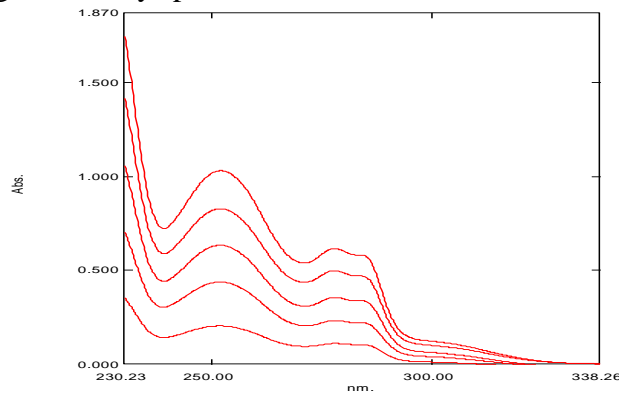


Fig.6 Overlay spectrum of Absorbance Ratio

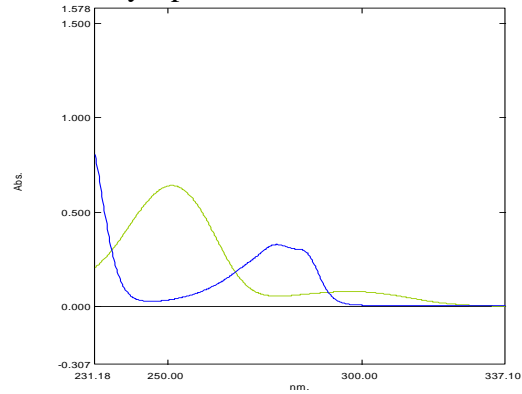


Fig.5 Overlay Spectrum of Mixture.

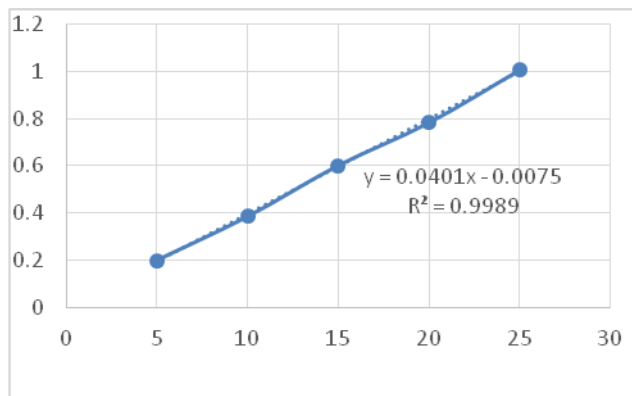


Fig.8 Calibration curve of DMF at 277nm nm by Simultaneous equation method

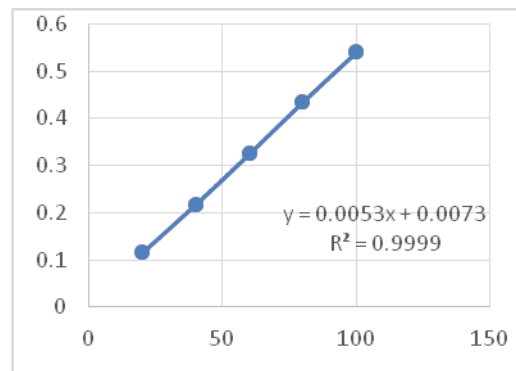


Fig.7 Calibration curve of BUP at 251 by Simultaneous equation method

by

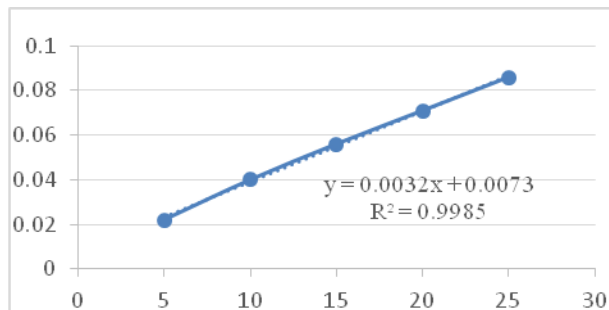


Fig.9 Calibration curve of BUP at 277 nm by Simultaneous equation method

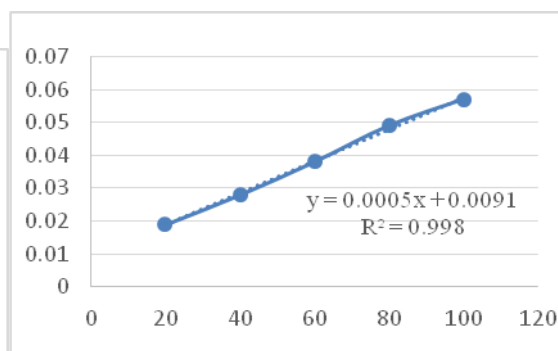


Fig.10 Calibration curve of DMF at 251 nm by Simultaneous equation method

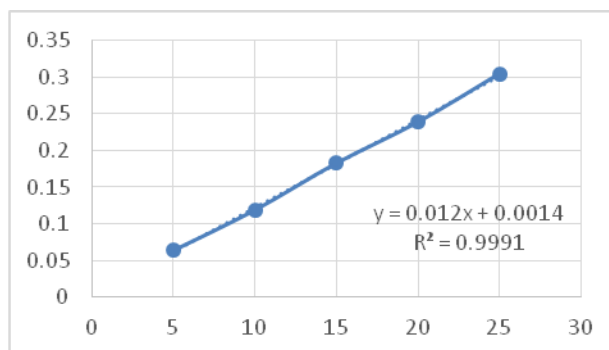


Fig.11 Calibration Curve of BUP at 267 nm by Absorption Ratio method.

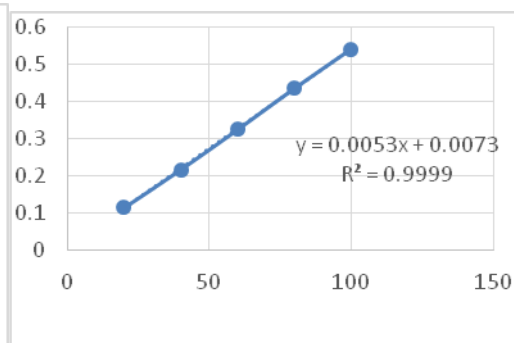


Fig.12 Calibration Curve of DMF at 277 nm by Q-Absorption Ratio method.

VALIDATION PARAMETER:

Validity of the proposed methods were confirmed by performing linearity, limit of detection, limit of quantification, accuracy, precision and stability studies as per the ICH guidelines.

Linearity

The calibration plot provides important information about the linearity of the proposed method. In the present study linearity range was studied in the concentration range of 5-25 µg/ml for BUP (Table 1 and Table 3 for Simultaneous equation method and Table 5 for Q-absorbance method). DMF exhibited excellent linearity in the range of 20 to 100 µg/ml (Table 2 and Table 4 for Simultaneous equation method and Table 6 for Q-absorbance method). The regression equations and regression coefficients are listed in Table 7.

TABLE 1: RESULTS OF CALIBRATION CURVES FOR BUP AT 251 nm BY SIMULTANEOUS EQUATION METHOD

Concentration ($\mu\text{g/ml}$)	Absorbance	%CV
5	0.2	1.5886
10	0.385	1.2246
15	0.601	1.2909
20	0.782	0.3406
25	1.005	0.2239

TABLE 2: RESULTS OF CALIBRATION CURVES FOR DMF AT 277 nm BY SIMULTANEOUS EQUATION METHOD

Concentration ($\mu\text{g/ml}$)	Absorbance	%CV
20	0.116	1.9337
40	0.218	0.7470
60	0.325	0.5331
80	0.435	1.0201
100	0.54	0.9374

TABLE 3: RESULTS OF CALIBRATION CURVES FOR BUP AT 277nm BY SIMULATENOUS EQUATION METHOD

Concentration ($\mu\text{g/ml}$)	Absorbance	%CV
20	0.022	0.670
40	0.04	0.6615
60	0.056	0.5248
80	0.071	0.5075
100	0.086	0.3462

TABLE 4: RESULTS OF CALIBRATION CURVES FOR DMF AT 251 nm BY SIMULATENOUS EQUATION METHOD

Concentration ($\mu\text{g/ml}$)	Absorbance	%CV
20	0.019	0.6756
40	0.028	1.4901
60	0.038	0.9221
80	0.049	0.8500
100	0.057	0.837

TABLE 5 : RESULTS OF CALIBRATION CURVES FOR BUP AT 267 nm BY Q-ABSORPTION RATIO METHOD.

Concentration (µg/ml)	Absorbance	%CV
5	0.064	1.3996
10	0.119	0.9103
15	0.183	0.5929
20	0.239	0.4613
25	0.305	0.4576

TABLE 6: RESULTS OF CALIBRATION CURVES FOR DMF AT 277 nm BY Q-ABSORPTION RATIO METHOD.

Concentration (µg/ml)	Absorbance	%CV
20	0.116	1.9337
40	0.218	0.7470
60	0.325	0.5331
80	0.435	1.0201
100	0.54	0.9374

Limit of detection and quantification limits

The detection and quantification limits were estimated using the linearity curve parameters. The LOD was calculated as 3.3 times the standard deviation of the intercept to the slope of the curve. The LOQ was 10 times the standard deviation of the intercept to the slope of the curve. The low LOD and LOQ values are tabulated in TableVII, indicating the good sensitivity of the proposed methods.

Precision

The precision of the established procedures was also assessed in terms of intra and inter-day by analyzing five concentrations of both analytes in the calibration curve range. For intra-day, solutions were analyzed six times in day and these solutions were investigated for three succeeding days for inter day precision. The percent relative standard deviation was calculated and presented in Table 7. The result showed low percent RSD, which confirmed that the proposed methods were precise.

Accuracy

Accuracy of the developed procedures were examined by assaying different concentration of both the analytes in the calibration concentration range. The accuracy of the methods was expressed in terms of the percent recovery and percent relative error. The mean percentage recovery was 99.83 % to 99.38 % for BUP and 99.75 % to 99.76 % for DMF tableI.

TABLE 7: VALIDATION PARAMETER RESULT OF THE PROPOSED SPECTROSCOPIC METHODS FOR SIMULTANEOUS DETERMINATION OF BUP AND DMF

Validation parameter	BUPROPION		DEXTROMETHORPHAN	
	Simultaneous Equation	Q-Absorbance Ration method	Simultaneous Equation	Q-Absorbance Ration method
Wavelength (nm)	251	267	277	277
Linearity range($\mu\text{g/ml}$)	5-25	5-25	20-100	20-100
Slope	0.0401	0.012	0.0053	0.0053
Intercept	0.0075	0.0014	0.0073	0.0073
Regression coefficient	0.9989	0.9991	0.9999	0.9999
LOD($\mu\text{g/ml}$)	0.2660	0.3135	1.7277	0.1666
LOQ($\mu\text{g/ml}$)	0.8062	0.95	5.2356	0.5030
Accuracy (Mean %)	99.83	99.38	99.75	99.76
Precision(%RSD)				
Intra day	0.5699	0.9712	0.6255	0.3222
Inter day	0.2710	0.7025	0.3500	0.4290

CONCLUSION:

A simple, accurate and reproducible UV spectrophotometric methods were established for concurrent quantification of BUP and DMF. Water has been used as a solvent, which makes these methods economical and eco-friendly. Analysis of laboratory prepared mixture confirmed the accuracy, specificity and also absence of interference from formulation excipients.

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ABBREVIATIONS

UV: Ultra Violet, %RSD: Percent Relative Standard Deviation; BUP: Bupropion, DMF: Dextromethorphan, %CV: Percent Co efficient of Variation.