Spectrophotometric estimation of eflornithine hydrochloride by using ion-pair reagents

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Abstract: Four newer, cost effective and sensitive ion-pair complex methods were estimated for the determination of eflornithine hydrochloride drug in pharmaceutical formulation. In these methods eflornithine hydrochloride react with bromocresol green (buffer of pH 4), bromophenol blue (buffer pH 4.5), methyl orange (buffer of pH 5.5) and bromothymol blue (buffer of pH 5) respectively. The chloroform was used for extraction of ion-pair complexes. The measurement of complexes was done at 413, 416, 417 and 425 nm respectively. Under the described conditions the proposed methods are linear over the concentration range of 3-18, 4-16, 6-30 and 2-12 µg/ml and the coefficient of determination were >0.999 (n=6) with a relative standard deviation of <1% (n=6). The average recovery of the target compound is >100% with a limit of quantification (LOQ) of 20, 0.869, 2 and 4.167 µg/ml and the limit of detection (LOD) 6.6, 0.287, 0.66 and 1.375 µg/ml. The mechanism of the derivatization reaction is proposed and advantages of the proposed method are discussed.

Keywords: DFMO hydrochloride; spectrophotometry; ion-pair formation; validation.

INTRODUCTION

Eflornithine hydrochloride (Difluoromethylornithine; DFMO) is a selective, irreversible inhibitor of ornithine decarboxylase enzyme, one of the key enzymes in the polyamine biosynthetic pathway (Merali and Clarkson, 1996). Eflornithine hydrochloride (DFMO) has the chemical name (2-fluoromethyl-DL-ornithine) (fig. 1).

DFMO is an antiprotozoal agent which is used for meningoencephalic stage of trypanosomiasis. It is occured by *Trypanosoma brucei gambienze*, African trypanosomiasis (Pepin *et al*., 1987; Merali and Clarkson, 1996). DFMO is a drug found to be effective in the treatment of facial hirsutism (Wolf *et al*., 2007) as well as in African trypanosomiasis, sleeping sickness (Pepin *et al*., 1987).

The methods available for the determination of DFMO included high pressure liquid chromatography techniques (Cohan *et al*., 1989; Huebert *et al*., 1997; Saravanan *et al*., 2009). The high pressure liquid chromatography methods available for the determination of eflornithine involve either pre or post column derivatization with UV or fluorescence detection (Kilkenny *et al*., 1998; Jannon-Lofmark *et al*., 2010) and LC carried out by evaporative light scattering detection (Malm and Bergqvist, 2007). Few analytical developed methods have been proposed for the determination of DFMO (Kumar *et al*., 2008; Kumar *et al*., 2009).

As per the literature survey, DFMO has been ion-pair associated with ion pairs reagents and determined by a ion-pair complex method for the first time. The developed methods for DFMO have many uses due to its time consuming, cost effective. The developed techniques ware validated for parameters such as linearity, accuracy. Precision, specificity and selectively, detection and quantification limits. The present research thus explains accurate and precise ion-pair complex methods for the estimation of DFMO.

MATERIALS AND METHODS

Materials and solutions

DFMO and 200 mg/ml vials (Ornidyl) were gifts from Wintac Limited (Bangalore, India). Bromophenol blue, methyl orange, bromothymol blue and bromocresol green were purchased from Merck (Darmstadt, Germany). All reagents used were of analytical grades. Distilled water was used.

Hydrochloric acids were supplied from Merck. Sodium hydroxide was supplied from Adwic (India), and Chloroform was supplied by Thermo Fisher Scientific Pvt. Ltd. (India, Mumbai).

Stock (1 mg/ml) solution of DFMO was prepared by dissolving the accurate weighed method. Further dilutions were done from stock solution to get desired concentrations.

Ornidyl (1 mg/ml) sample solution (Label claim, each fill volume of the vial contains 100 ml and each ml contains 200 mg) was prepared by pipetting out 10 ml of the contents of the vial and geometrically diluted suitably

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with double distilled water to get the required concentration.

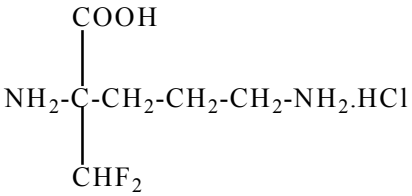

Fig. 1: DFMO hydrochloride (DFMO).

Acetate buffer solution was prepared using the recommended method by Indian Pharmacopeias (Controller, 1996).

Citrate buffer was prepared by dissolving the accurate weight (0.6 g) of citric acid and (0.39 g) of disodium hydrogen phosphate in 90 ml double distilled water, pH adjusted to 2-6 with citric acid and made up to 100 mL.

0.1 % solution of all reagents was prepared by dissolving 100 mg of BPB, BCG, BTB and MO in 100 ml citrate buffer solution pH 4.5, 4, 5 and 5.5 respectively.

Instrument

A Shimadzu (UV-160A) UV spectrophotometer with 1 cm quartz cells was used.

Determination of DFMO using bromophenol blue (BPB)

Different volumes of (1 mg/ml) of DFMO (0.15-0.9 ml) were taken into a 50 ml separating flask. Double distilled (25ml) water and 0.1 % (12 ml) of BPB solution in citate buffer of pH 4.5 were added respectively. After the reaction, solution was stand for 10 min of time for complex formation, the ion-pairs were extracted with 3 times with 10 ml of chloroform. Now the chloroform layer was passed through the anhydrous sodium sulphate (previously moistened with chloroform). The final volume was adjusted to 50 ml using chloroform. The measurement of the formed ion-pair was at 413 nm (fig. 2).

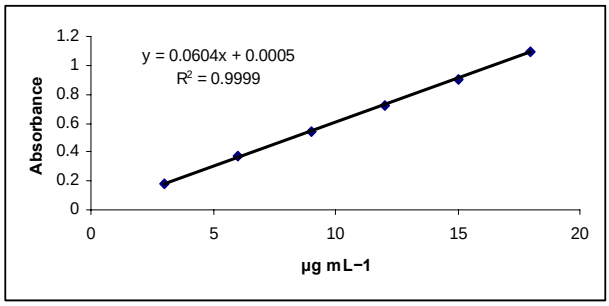

Fig. 2: Calibration curve of DFMO-BPB using pH 4.5 of citrate buffer.

Procedure for the parenteral formulations

To a definite volume of ornidyl, equivalent to 0.1 g of DFMO was transferred to a 100 ml volumetric flask and made up to 100 ml of distilled water to get (1 mg/ml). The above mentioned procedure was followed in the range of (3-18 µg/ml). The drug concentrations were calculated from the standard calibration graph.

Determination of DFMO using bromocresol green (BCG)

Different volumes of (1 mg/ml) of DFMO (0.2-0.8 ml) were transferred into a 50 ml separating funnel. 25 ml of distilled water and 15 ml of 0.1 % (w/v) of BCG solution in citrate buffer pH 4 were added respectively. After the reaction, the mixture was allowed to stand for 10 min of time for complex formation, the ion-pairs were extracted with three times with 10 ml portions of chloroform. The chloroform layer was passed through the anhydrous sodium sulphate (previously moistened with chloroform). The volume was adjusted to 50 ml using chloroform. The calibration curves were constructed at 416 nm using BCG reagent (fig. 3), against a blank solution.

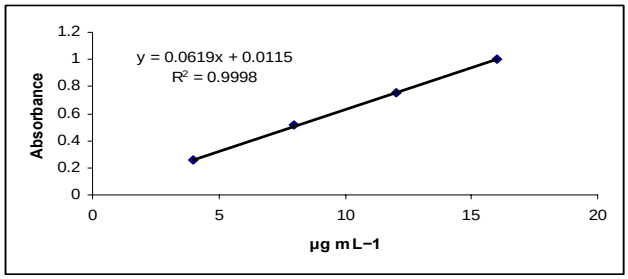

Fig. 3: Calibration curve of DFMO-BPB using citrate buffer solution of pH 4.

Procedure for the parenteral formulations

To an accurately measured volume of ornidyl vial, equivalent to 0.1 g of DFMO hydrochloride was taken into a 100 ml volumetric flask and made up to 100 ml with distilled water to get (1 mg/ml). The above mentioned procedure was followed in the linear range of (4-16 µg/ml) the drug concentrations were calculated from the standard calibration graph prepared under prescribed conditions.

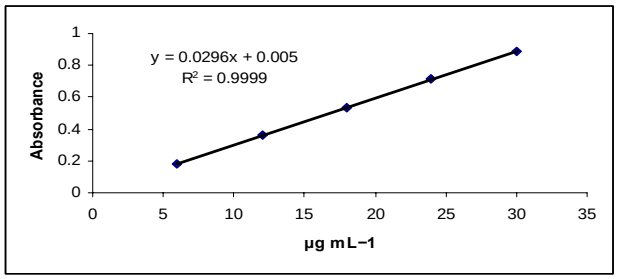

Fig. 4: Calibration curve of DFMO-BPB using pH 5 of citrate buffer.

Determination of DFMO using bromothymol blue (BTB)

Different volumes of (1 mg/ml) of DFMO (0.3-1.5ml) were transferred and add 25 ml of distilled water and 16

ml of 0.1% BTB solution in citate buffer of pH 5 were added respectively. After the reaction mixture was allowed to stand at room temperature for 10-15 min of time for complex formation, the ion-pairs were extracted. The chloroform layer was passed through the sodium sulphate (previously moistened with chloroform). The measured volume was adjusted to 50 ml with chloroform. Now measured the absorbance of the formed ion-pair at 417 nm (fig. 4) against a blank solution.

Procedure of the parenteral formulations

To an specific volume of ornidyl sample, (0.1g) of DFMO hydrochloride was transferred to a 100 ml volumetric flask and made up to 100 ml with water to get (1 mg/ml). The above mentioned procedure was followed in the range (6-30µg/ml). The drug concentrations were calculated from the standard linearity graph.

Determination of DFMO by methyl orange (MO)

Different volumes of (1 mg/ml) of DFMO (0.1-0.6 ml) were transferred into a 50 ml separating funnel. 25 ml of double distilled water and 18 ml of 1 % (w/v) of MO solution in citate buffer of pH 5.5 were added respectively. After the reaction, the solution was allowed to stand for 10-20 min of time for complex formation. The ion-pairs were extracted with three times with chloroform. The volume was adjusted to 50 ml using chloroform. The calibration curves were constructed at 425 nm using MO reagent (fig. 5), against a blank solution.

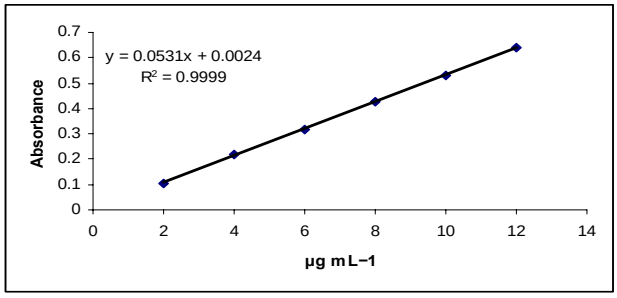

Fig. 5: Calibration curve of DFMO-BPB using citrate buffer of pH 5.5.

Procedure for the parenteral formulations

To an accurately measured volume of ornidyl, equivalent to (0.1 g) of DFMO hydrochloride was transferred to a 100 ml volumetric flask and made upto 100 ml with double distilled water to get (1 mg/ml). The above mentioned procedure was followed in the range of (2-12 µg/ml). The drug concentrations were calculated.

RESULTS

Absorption spectra

The absorption spectra of the proposed methods (413, 416, 417 and 425 nm) were shown in (fig. 6).

Effect of pH

The pH effect was investigated over the acidic pH range 2-6 using different types of buffer solutions (citrate, phthalate and phosphate). Citrate buffer is the best for complex formation (fig. 7) and to the stability of the colour without affecting the result.

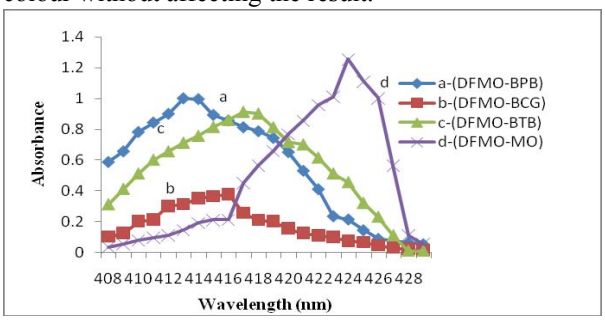

Fig. 6: Absorption spectra of all methods: (a) DFMO-BPB ion pair complex (12 µg/ml); (b) DFMO-BCG ion pair complex (8 µg/ml); (c) DFMO-BTB ion pair complex (15 µg/ml); (d) DFMO-MO ion pair complex (8 µg/ml)

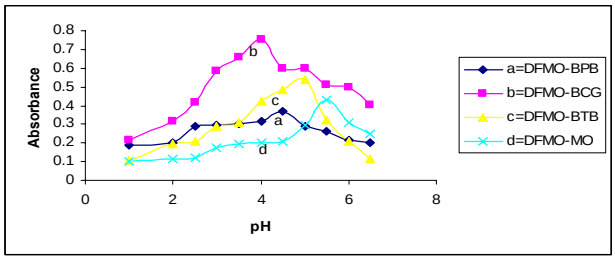

Fig. 7: Effect of pH on the absorbance of DFMO hydrochloride.

Effect of concentration of reagent

The reagent concentration effect on the drug was investigated over the different range 0.01-0.1 % (w/v) using citrate buffer (fig. 8).

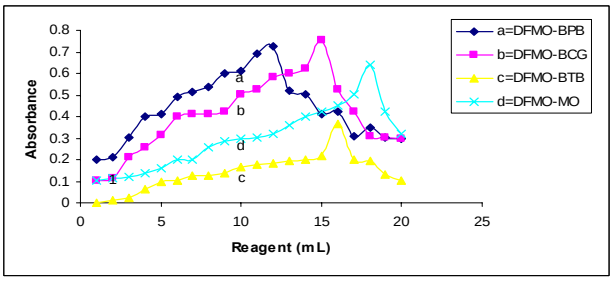

Fig. 8: Effect of ion-pair reagents on the absorbance of DFMO hydrochloride.

Effect of reaction time (complexes formation) and temperature

The optimum reaction time was investigated at ambient temperature (25±2°C). Maximum chromogen was attained after 10-15 min of mixing.

Stability study of ion-pair colour complex

Stability studies of the colored chromogen were developed and show that color was stable for more than 1 h at room temperature (fig. 9).

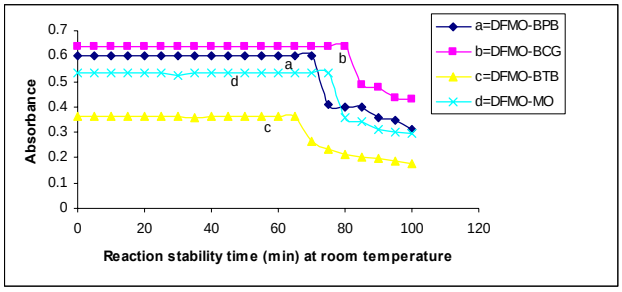

Fig. 9: Stability study for ion-pair color complexes.

Stoichiometry of the formed ion-pairs complex

The structure of charge transfer complex formed between the drug and reagents shown in (figs. **10-11**).

Method validation and Optical Characteristics

Validity of Beer's law

The optical characteristics of the proposed methods have been calculated. The values are given in table 1.

The result of mean relative error (RME), relative standard deviation (RSD) and recoveries by the proposed methods are shown in table 2.

Table 1: Optical characteristics of ion-par complex methods

Parameter	DFMO-BPB	DFMO-BCG	DFMO-BTB	DFMO-MO
λmax (nm)	413	416	417	425
Beer's law limit (µg/ml)	3-18	4-16	6-30	2-12
Molar absorptivity (L/mol/cm^{-1})	0.0605	0.0632	0.0299	0.0535
Sandell's sensitivity (µg cm^{-2})	0.0166	0.0158	0.0335	0.0159
Regression equation* Y= mC+b				
Intercept (b)	0.0604	0.0619	0.0296	0.0531
Slope (m)	0.0005	0.0115	0.005	0.0024
Correlation coefficient** (r)	0.9999	0.9998	0.9999	0.9999
LOD (µg/ml)	6.6	0.287	0.66	1.375
LOQ (µg/ml)	20	0.869	2.0	4.167
SD	0.001	0.001	0.001	0.001
RSD (%)	0.23-0.40	0.21-0.45	0.25-0.54	0.38-0.52
Percentage recovery (%)	99.22-102.33	100.20-102.25	100.28-102.50	100.25-103.75

* Y= mC+b, where Y is the absorbance and b is the concentration of the drug in µg/ml.
** Average of six determinations.

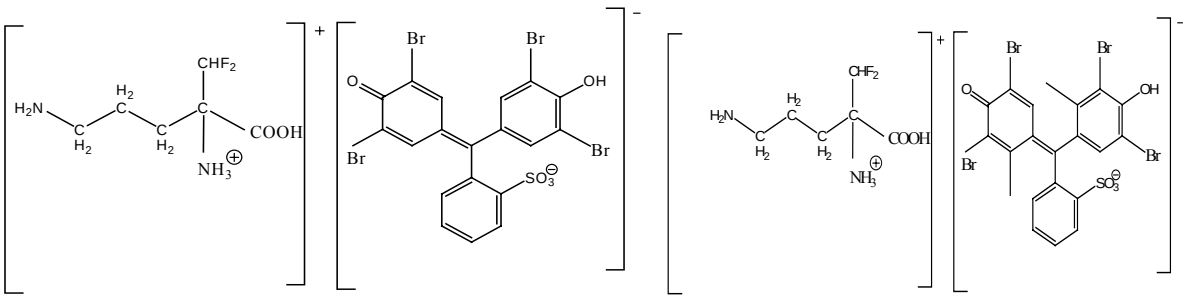

Fig. 10: Structure of DFMO-BPB and DFMO-BCG ion-pairs complex.

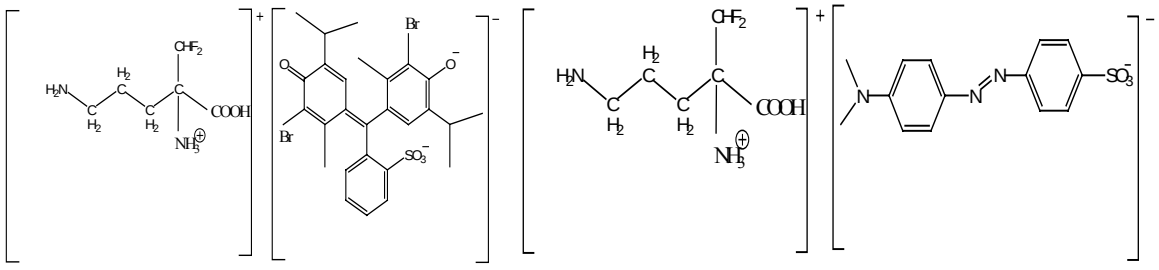

Fig. 11: Structure of DFMO-BTB and DFMO-MO ion-pairs complex.

Table 2: Intra-day and inter-day precision and accuracy of DFMO proposed methods.

Methods	Added concentration (µg/ml)	*Found concentration (µg/ml)	Percentage recovery	RME (%)	RSD (%)
Intra-day					
BPB	3.0	3.04	101.33	1.33	0.48
	6.0	6.05	100.83	0.83	0.35
	9.0	8.93	99.22	0.78	0.28
	12.0	12.02	100.17	0.17	0.22
BCG	4.0	4.03	100.75	0.75	0.56
	6.0	6.05	100.83	0.83	0.42
	8.0	8.05	100.63	0.63	0.38
	10.0	10.02	100.20	0.20	0.25
BTB	6.0	6.10	101.67	0.67	0.65
	10.0	10.08	100.80	0.80	0.54
	14.0	14.07	100.50	0.50	0.45
	18.0	18.05	100.28	0.28	0.34
MO	2.0	2.01	100.50	0.50	0.58
	4.0	4.09	102.25	0.25	0.50
	6.0	6.07	101.17	0.17	0.46
	10.0	10.06	100.60	0.60	0.39
Inter-day					
BPB	3.0	3.07	102.33	2.33	0.65
	6.0	6.09	101.50	1.50	0.46
	9.0	9.01	100.11	0.11	0.42
	12.0	12.08	100.67	0.67	0.35
BCG	4.0	4.09	102.25	2.25	0.68
	6.0	6.10	101.67	1.67	0.55
	8.0	8.11	101.37	1.37	0.48
	10.0	10.09	100.90	0.90	0.32
BTB	6.0	6.15	102.50	2.50	0.72
	10.0	10.06	101.60	1.60	0.66
	14.0	14.09	100.64	0.64	0.52
	18.0	18.08	100.44	0.44	0.45
MO	2.0	2.05	102.50	2.50	0.67
	4.0	4.15	103.75	3.75	0.53
	6.0	6.14	102.33	2.33	0.58
	10.0	10.11	101.10	1.10	0.46

* Mean value of four determinations in six replicates. RME- Relative mean error; RSD- Relative standard deviation

DISSCUSION

The absorption spectra of the proposed methods were found to be 413, 416, 417 & 245 nm. The absorbance of the ion-pairs complexes show a maximum using citrate buffer solution at pH 4.5, 4, 5 and 5.5 for DFMO-BPB, DFMO-BCG, DFMO-BTB and DFMO-MO respectively. The maximum and constant absorbance of ion-pair complex formation is obtained at pH 4.5 of 0.1 % (w/v) BPB of 12 ml, pH 4 of 0.1 % (w/v) BCG of 15 ml, pH 5 of 0.1 % (w/v) BTB 16 ml and pH 5.5 of 0.1 % (w/v) MO of 18 ml for DFMO-BPB, DFMO-BCG, DFMO-BTB and DFMO-MO respectively. The stability of color chromogen was studied. The color chromogen of proposed methods was stable up to 1 hour.

In all four methods, DFMO is determined via ion-pair charge transfer complex formation reaction which occurred between the drug (electron acceptor) and reagents (electron donar). The optimum complex formation is obtained at acidic pH 4.5 4, 5 and 5.5 for DFMO-BPB, DFMO-BCG, DFMO-BTB and DFMO-MO. Concentration effect on reagents on ion-pair coloured product was studied.

Under the selected conditions, a linear relationship between the absorbance A of DFMO-reagent derivative chromogen and the concentration C of DFMO is obtained in the range of 3-12, 4-16, 6-30 and 2-12 µg/ml for all methods. Calibration graphs were constructed by measuring the absorbance at six concentration levels which showed linear response of absorbance. The precision of the proposed methods was studied by determination of the drug in six replicates, individually derivatized with reagents at different concentration obtaining relative standard deviations of <1% (table 2). Accuracy was determined as the mean relative error (RME%) and Precision was showed as the relative standard deviation (RSD%).

Table 3: Results of recovery studies by standard addition method*

Added concentration (µg/ml)	Recovery ± RSD%
BPB	
3.0	99.42±0.39
6.0	100.23±0.21
9.0	99.11±0.21
12.0	98.87±0.33
BCG	
4.0	98.67±0.24
6.0	98.88±0.19
8.0	100.72±0.14
10.0	100.11±0.11
BTB	
6.0	100.02±0.65
10.0	100.13±0.57
14.0	100.17±0.48
18.0	100.23±0.32
MO	
2.0	100.12±0.71
4.0	99.54±0.68
6.0	99.84±0.56
10.0	99.58±0.51

* Mean value of four determinations in six replicates.

CONCLUSION

The data given above reveal the proposed methods for DFMO determination has many advantages. Methods are simple and quick. Their linear ranges are relatively wide and do not need expensive apparatus. Furthermore, the proposed methods may be successfully used to determine DFMO in pharmaceutical formulation. Accordingly, the methods are practical and valuable.

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