International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 3, Issue 3, 2011

Research Article

SPECTROPHOTOMETRIC AND SPECTROFLUORIMETRIC DETERMINATION OF MEMANTINE HYDROCHLORIDE IN BULK AND PHARMACEUTICAL PREPARATIONS

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Received: 01 April 2011, Revised and Accepted: 02 May 2011

ABSTRACT

In the current work, we present three simple, sensitive and robust methods for the determination of memantine hydrochloride in pure and dosage form. The first method is based on the reaction of the drug with 4-chloro-7-nitro-2,1,3-benzoxadiazole in alkaline buffer. The product formed is measured spectrophotometrically at 476 nm or spectrofluorimetrically in acetone at 500 nm after excitation at 455 nm (second method). The third method investigates the reaction of the analyte with *o*-phthalaldehyde/ N-acetyl-L-cysteine and the absorbance of the produced derivative is measured at 340 nm. Linearities were displayed over the concentration ranges of 5-70, 0.02-0.2, 5-50 µg/ml for all methods, respectively. The proposed methods were validated in terms of accuracy, precision, robustness and limits of detection and quantitation were calculated. Commercially available tablets were successfully analyzed by the developed procedures. The results were in good agreement with that obtained from the reference method.

Keywords: Memantine hydrochloride, Derivatization, Spectrophotometry, Spectrofluorimetry, Dosage form

INTRODUCTION

Memantine hydrochloride (MEM) is a 1-amino-3,5-dimethyladamantane derivative that acts as a low affinity, uncompetitive NMDA receptor antagonist. In the late 80's, it was launched by German Merz Co. for the treatment of dementia[1,2]. Recently MEM was FDA approved for the treatment of moderate to severe Alzheimer's disease[2]. As predicted, MEM exceeded the blockbuster mark of 1 billion US dollars in 2007. Moreover, researches demonstrated the potential of its efficacy in the treatment of other neurological disorders including Parkinson's disease, spasticity[1], pervasive developmental disorders[3], schizophrenia, alcohol abuse and withdrawal[4].

The tricyclic saturated amino compound lacks any significant UV or fluorescence properties. Therefore, methods including gas chromatography- mass spectrometry GC-MS[5,6], high performance liquid chromatography (HPLC) coupled to MS and HPLC- tandem mass spectrometry (MS/MS) were used for its determination[7-10]. In addition, MEM was determined by capillary zone electrophoresis (CZE)[11] and recently by micellar electrokinetic chromatography with laser induced fluorescence (MEKC-LIF)[12]. Furthermore, derivatization of its primary amino group followed by liquid chromatographic separation of the formed adduct has been implemented in a variety of biological matrices. For this purpose, the use of derivatizing reagents such as; 2-naphtoxy acetyl chloride[13,14], anthraquinone-2-sulfonyl chloride[15], 9-fluorenylmethyl chloroformate[16] and dansyl chloride was reported[17,18]. A liquid chromatographic method with fluorimetric detection using 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F) was developed for the simultaneous assay of the drug and its analogs. However, this method was of comparable lower sensitivity to MEM[19]. *o*-Phthalaldehyde (OPA) was used for the liquid chromatographic determination of its prototype amantadine hydrochloride in plasma[20,21], yet it has not been reported for the determination of MEM.

In spite of the effectiveness of the aforementioned techniques, they are more suitable for MEM determination in complex matrices as biological fluids. However, simpler and lower cost alternatives are favored for the quality control (QC) of the drug in commercial preparations where a greatly simpler matrix is employed. Among such techniques are the spectrophotometric methods which have generally been the methods of choice for routine analysis in industrial QC laboratories, especially in developing countries. Very few methods were recently published for MEM quantitation in tablets. Such methods have used different acidic dyes as well as 1,2-napthaquinone-4-sulphonate[22,23]. Nevertheless, these procedures though easy, they were lengthy because of an additional liquid-liquid extraction step of the formed derivative prior to measurements. Therefore, the need for simple, rapid and sensitive methods for the quantitation of MEM in tablets becomes obvious.

MEM is formulated as a 10 mg tablet and as a 10 mg/g oral drops solution; only the former is available on our local market and was subsequently used in the current work. To our best knowledge, neither 4-chloro-7-nitro-2,1,3-benzoxadiazole (NBD-Cl) nor OPA has been previously used for the direct spectrophotometric or spectrofluorimetric determination of MEM in its tablet matrix. The aim of this work is to develop simple, sensitive and robust methods for the quantification of MEM which could be adopted for its routine quality control in tablet formulations.

MATERIALS AND METHODS

Apparatus

Helios α UV-VIS spectrophotometer with 10-mm matched quartz cells was used for all spectrophotometric measurements. Spectra were obtained through Vision 32 Software. Absorption wavelengths were set at 476 and 340 nm for NBD-Cl and OPA methods, respectively.

Spectrofluorimetric measurements were carried out on a Perkin-Elmer Model LS 45 Luminescence Spectrometer with 10-mm quartz cell and interfaced to a personal computer loaded with FL Win lab Software. Excitation and emission wavelengths were set at 455 and 500 nm, respectively for the NBD-Cl method.

Chemicals and reagents

All reagents and solvents were of analytical grade. MEM was a gift from Adwia Co. (Cairo, Egypt). NBD-Cl (≥ 99%), OPA (≥98.5%) were purchased form Fluka (Buchs, Switzerland) and prepared in methanol at 5 and 4 mg/ml, respectively. N-acetyl-L-cysteine (NAC) (≥ 99%) was obtained from Sigma-Aldrich (MO, USA) and prepared as a 5 mg/ml solution in water. For the preparation of borate buffer 0.5 M pH 8.6 and 0.2 M pH 9.6, proper weights of boric acid and KCl were dissolved in 100 ml water; pH value of each buffer was adjusted with the NaOH solution of corresponding molarity and finally the volume was completed to 200 ml with water[24]. 2 M HCl was used for the acidification in NBD-Cl method. Ebixa® (Lundbeck Ltd., Copenhagen, Denmark, 10 mg/tablet), Memexa® (Copad Pharma, Cairo, Egypt, 10 mg/tablet) and Ravamantine® (Eva Pharma, Giza, Egypt, 10 mg/tablet) tablets were purchased from the local market. Distilled water was used throughout the work.

Standard solutions

MEM stock solutions of 1 and 0.5 mg/ml were prepared by dissolving the proper weights of the drug in methanol and water for the NBD-Cl and OPA methods, respectively.

Procedure

Calibration curves

NBD-Cl method

For the spectrophotometric measurements, serial volumes (0.05 - 0.7 ml) of MEM standard solution were transferred into a set of 10 ml standard volumetric flasks and the volumes were adjusted to 1 ml with methanol. To each flask, 1 ml of 0.5 M borate buffer pH 8.6 was added followed by 1 ml of NBD-Cl reagent. The reaction mixtures were heated in a thermostatically controlled water bath maintained at 90 °C for 25 min and then they were cooled under tap water. Afterwards, 1 ml of 2 M HCl was added to each flask, and the volume was completed to the mark with methanol. The absorbances of the colored solutions were measured at 476 nm against a reagent blank treated similarly, and calibration curves were constructed.

For the spectrofluorimetric measurements, 0.02 ml of the previously derivatized solutions were transferred into another set of 10 ml volumetric flasks and diluted up to the mark with acetone. Excitation and emission wavelengths were set at 455 and 500 nm, respectively.

OPA method

Serial volumes (0.1 - 1 ml) of MEM standard solution were transferred into a set of 10 ml standard volumetric flasks and the volumes were adjusted to 1 ml with water. 2 ml of 0.2 M borate buffer pH 9.6 were added to each flask followed by 0.6 ml of NAC and OPA reagents, respectively. The reaction mixtures were left for 15 min at room temperature and then the volumes were completed to the mark with water. The solutions were measured at 340 nm against a reagent blank treated similarly.

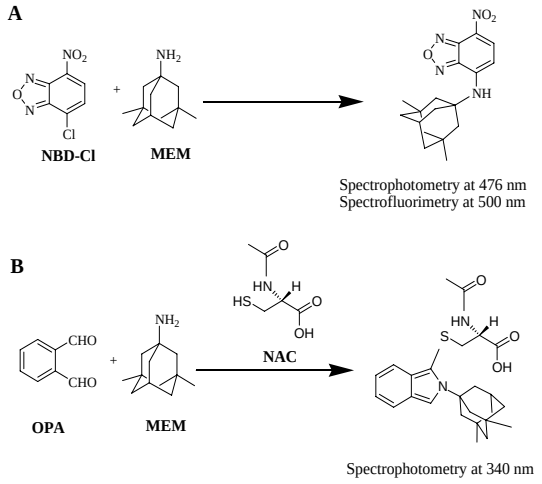

Fig. 1: The proposed reaction pathway between MEM and (A) NBD-Cl, (B) OPA

Assay of pharmaceutical preparations

Twenty one tablets, each labeled to contain 10 mg, were accurately weighed and powdered. Of the powdered tablets, weights equivalent to 50 and 25 mg MEM were transferred into 50 ml volumetric flasks for the NBD-Cl and OPA methods, respectively. To each flask 40 ml of either methanol (NBD-Cl method) or water (OPA method) was added followed by sonicating both solutions for 15 min. Subsequently, the extracts were filtered into another set of 50 ml flasks. Finally, the filtrates were completed to the mark with either methanol or water as above to yield 1 and 0.5 mg/ml MEM solutions. The nominal contents of tablets were then calculated using the corresponding regression equation.

RESULTS AND DISCUSSION

Optimization of the reaction conditions

NBD-Cl method

This benzoxadiazole reagent is a highly sensitive chromogenic and fluorogenic reagent used for the derivatization of both primary and secondary amines[25,26]. MEM reacts with NBD-Cl in alkaline medium to give a yellow colored product (Fig. 1.A) which reads in the visible range at λ_{max} 476 nm (Fig. 2.A) and fluoresces in acetone at 500 nm when excited at 455 (Fig. 2.B). Different experimental parameters were investigated to optimize the derivatization reaction. Importantly, the volume of the aqueous medium was found to compromise the absorbance of the formed derivative significantly. Therefore, MEM stock solution was prepared in methanol. In addition, the effect of buffer volume on the derivatization yield was studied, and a volume of 1 ml was considered appropriate (Fig. 3). Buffer strength and pH were varied to study their effect; maximum color intensity was obtained at a buffer concentration of 0.5 M and a pH of 8.6 (Fig. 4). As the reaction is kinetically catalyzed, different heating temperatures and time intervals were investigated. Heating the reaction mixture at 90 °C (Fig. 5) for 25 min gave the best result. With respect to the reagent volume, 1 ml of NBD-Cl was sufficient for a stoichiometric and complete reaction and larger volumes showed no further enhancement of the measured response. Prior to the photometric measurements, acidification of the medium to below pH 1 using 1 ml of 2 M HCl was crucially important since NBD-OH, the alkaline hydrolytic product of NBD-Cl, has a maximum absorbance at 460 nm[27].

A variety of solvents such as water, ethanol, methanol and acetone were tested as dilution media for the fluorimetric determination of the drug. The fluorescence response was greatly diminished upon using water or methanol for dilution. On the other hand, the highest fluorescence quantum yield was attained upon using acetone or ethanol as the diluting solvents. However, using ethanol, the fluorescence of the blank increased dramatically compromising the sensitivity of the method and therefore it was excluded.

In order to investigate the stability of the derivative, the reaction solutions were left at room temperature and the photometric measurements were carried out periodically. The colored adduct was found stable for at least 3 h after which the absorbance and fluorescence intensities start to fade slowly; a period long enough allowing the processing of large number of samples.

OPA method

This reagent reacts only with primary amines in the presence of an SH-donor compound[28]. NAC is the most favored among different thiols due to the stability of its adduct with OPA/primary amine[29, 30]. In addition, NAC lacks the unpleasant odor of other free thiols. The product of the reaction of MEM and OPA in the presence of NAC (Fig. 1.B) showed a maximum absorbance at 340 nm (Fig. 2.C). In order to optimize this reaction, the effect of borate buffer was studied; different buffer volumes, strengths and pH values were tried. 2 ml of 0.2 M borate buffer pH 9.6 (Fig. 3 and 4) were adequate. At room temperature, an apparently complete reaction needed 15 min .The effects of OPA:NAC molar ratio and OPA volumes were investigated. A volume of 0.6 ml OPA reagent and a 1:1 molar ratio (Fig. 6) were satisfactory and resulted in the highest absorbance intensity. Measuring the absorbance of the colored product at different time intervals revealed that it was stable for 4 h.

Method validation

Linearity and limits of detection and quantitation

For the proposed methods, series of six concentrations of MEM standard solutions prepared in triplicates were derivatized as previously described. The calibration curves were then constructed by plotting the measured response as a function of the corresponding concentration and the linearities for all methods

were calculated using the least square regression model. In all cases, high values of the correlation coefficients of the regression equations were obtained indicating a satisfactory linearity over each concentration range. Other important statistical parameters as standard deviation of the residuals ($S_{y/x}$), standard deviation of the intercept (S_a) and standard deviation of the slope (S_b) were also calculated. Small values obtained by these parameters indicate the negligible scatter of the experimental points around the fitted regression lines.

Limits of detection (LOD) and quantitation (LOQ) were calculated as 3.3 σ/S and 10 σ/S, respectively; where σ = standard deviation of the blank and S = slope of the calibration curve[31]. The data compiled in Table 1 summarize the characteristics of the calibration curves.

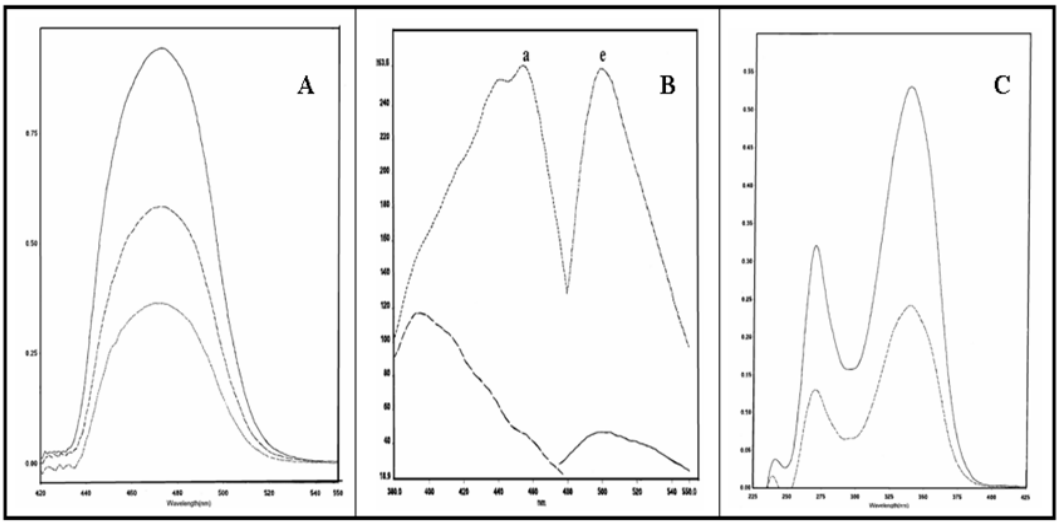

Fig. 2: ***(A)*** **Absorption spectra of the reaction product of MEM (20, 30, 50 µg/ml) with NBD-Cl** ***(B)*** **Absorption (a) and emission (e) spectra of the reaction product of MEM (0.06 µg/ml) with NBD-Cl against background.** ***(C)*** **Absorption spectra of the reaction product of MEM (10, 20 µg/ml) with OPA**

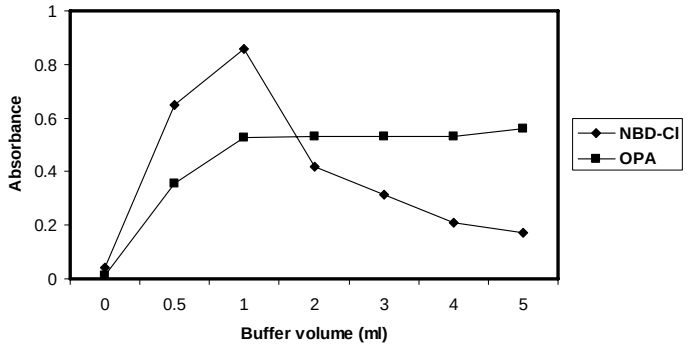

Fig. 3: Effect of buffer volume on the absorbance of the reaction product of MEM (40 µg/ml) with NBD-Cl and MEM (20 µg/ml) with OPA

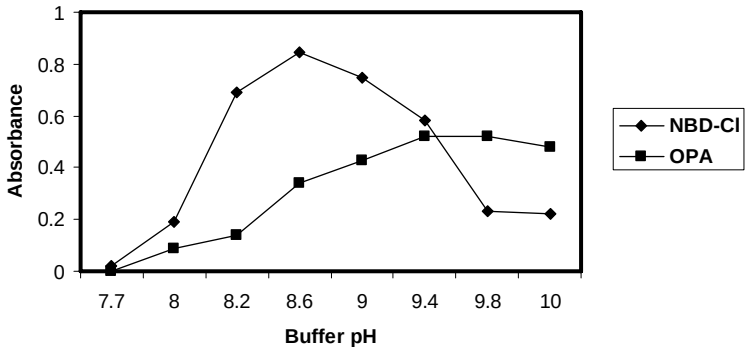

Fig. 4: Effect of buffer pH on the absorbance of the reaction product of MEM (40 µg/ml) with NBD-Cl and MEM (20 µg/ml) with OPA

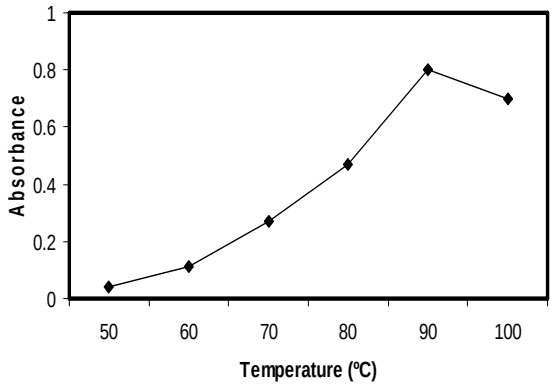

Fig. 5: Effect of temperature on the absorbance of the reaction product of MEM (40 µg/ml) with NBD-Cl

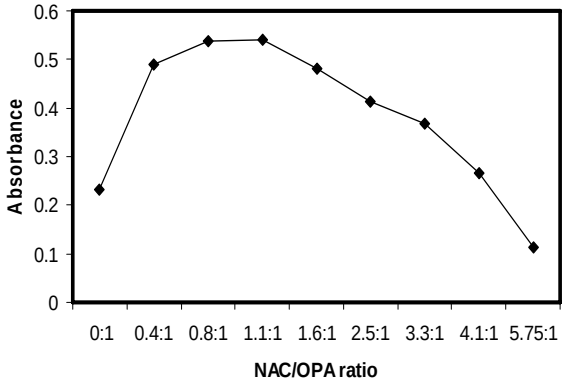

Fig. 6: Effect of NAC:OPA molar ratio on the absorbance of their reaction product with MEM (20 µg/ml)

Table 1: Quantitative parameters for the reaction of MEM with NBD-Cl and OPA

Parameter	NBD-Cl		OPA
	Spectrophotometry	Spectrofluorimetry	
Linear range (µg/ml)	5-70	0.02-0.2	5-50
Correlation coefficient	0.9999	0.9999	0.9994
Intercept	-0.0028	0.0436	-0.008
Slope	0.0212	3757	0.0272
S_a	4.3×10^{-3}	2.79	1.7×10^{-2}
S_b	1.1×10^{-4}	26.56	5.5×10^{-4}
$S_{y/x}$	6.3×10^{-3}	3.88	2.1×10^{-2}
LOD (µg/ml)	1.101	0.006	0.858
LOQ (µg/ml)	3.336	0.019	2.600

Precision and accuracy

In order to validate each method, quality control samples prepared at 3 concentration levels (low, medium and high) were analyzed as previously described on 3 consecutive days. The accuracy of each method was expressed as percentage relative error (Er %), while intra- and inter-day reproducibilities were calculated as relative standard deviations (RSD %). The proposed methods are deemed accurate and precise as demonstrated by the results gathered in Table 2.

Robustness and ruggedness

The robustness of the developed methods was tested by examining the influence of small but deliberate variations of the reaction conditions; these include the buffer pH, reagent concentration and reaction time. In each case only one parameter was changed while all other conditions were kept constant. Table 3 shows that these small variations did not significantly affect the suggested assays indicating their reliability during routine analyses. Ruggedness was also tested by applying the proposed methods as previously described but using two different spectrophotometers at two different laboratories. Results of lab-to-lab variations were reproducible (RSD ≤2%).

Analysis of pharmaceutical preparations

Commercially available MEM tablets were subjected to analysis by the proposed methods alongside with a reference method[23]. The former were selective for MEM determination with no observed interference from tablet excipients. Results of MEM recovery obtained by the suggested methods were satisfactory with standard deviations (SD) less than 1.2. Statistical analysis of the results obtained by the proposed and the reference assays revealed no significant difference regarding accuracy (*t*-test) and precision (*F*-test) at 95% confidence level (Table 4).

Table 2: Intra- and inter-day precision and accuracy of the reaction of MEM with NBD-Cl and OPA

Frequency of analysis	Method	Concentration added (µg/ml)	Concentration found ± SD (µg/ml)	RSD (%)	Er (%)
Intra day (n=5)	**NBD-Cl**	15	15.07±0.03	0.22	0.47
	Spectrophotometry	30	30.30±0.01	0.04	0.98
		55	55.52±0.31	0.56	0.94
		0.03	0.03±0.24	0.75	0.94
	Spectrofluorimetry	0.10	0.10±0.61	0.61	-0.44
		0.18	0.18±0.43	0.24	0.67
		15	15.05±0.17	1.15	0.35
	OPA	30	30.63±0.15	0.48	-1.15
		45	45.39±0.22	0.49	0.85
Inter day (n=5)	**NBD-Cl**	15	14.84±0.18	1.20	-1.05
	Spectrophotometry	30	30.04±0.33	1.12	0.14
		55	54.52±0.53	0.97	-0.87
		0.03	0.03±0.34	1.13	-0.57
	Spectrofluorimetry	0.10	0.10±0.75	0.76	-1.12
		0.18	0.18±0.43	0.24	-0.14
		15	14.80±0.12	0.79	-1.30
	OPA	30	29.99±0.30	1.00	-0.02
		45	44.98±0.43	0.96	-0.05

Table 3: Robustness of the proposed NBD-Cl and OPA methods for the determination of MEM

Parameters	% Recovery±RSD (%)[a]
NBD-Cl	
Recommended Conditions [b]	100.26±0.03
Heating Time (min)	
20	99.01±0.14
30	99.92±0.15
Buffer pH	
8.4	98.40±0.08
8.8	99.21±0.11
Reagent Concentration (mg/mL)	
4	98.62±0.10
6	100.31±0.04
OPA	
Recommended Conditions [b]	100.28±0.02
Reaction Time (min)	
10	99.69±0.25
20	100.00±0.01
Buffer Volume (mL)	
1.8	98.43±0.31
2.2	100.12±0.07
Buffer pH	
9.4	99.91±0.14
9.8	100.60±0.04
Reagent Concentration (mg/mL)	
3	99.13±0.03
5	100.09±0.12

[a] Each value is the average of 3 determinations; [b] Conditions are mentioned under *calibration curves*

Table 4: Statistical evaluation of the results obtained by the proposed methods for the determination of MEM in tablets

Statistical parameter	NBD-Cl		OPA	Reference method [a]
	Spectrophotometry	Spectrofluorimetry		
Ebixa tablet [b]				
Mean recovery (%) ± SD	100.11±0.47	100.08±1.2	99.68±0.77	99.75±0.94
t-test [c]	0.75	0.50	0.13	
F-test [c]	3.89	1.42	1.46	
Memexa tablet [b]				
Mean recovery (%) ± SD	99.46±0.84	99.70±0.45	99.35±0.78	99.29±0.78
t-test [c]	0.33	1.01	0.12	
F-test [c]	1.17	2.94	1.00	
Ravemantine tablet [b]				
Mean recovery (%) ± SD	99.60±0.87	99.21±0.61	99.74±0.63	99.49±0.92
t-test [c]	0.17	0.58	0.48	
F-test [c]	1.13	2.27	2.17	

[a] as described by Praveen[23; b] Each tablet is labeled to contain 10 mg MEM; [c] Theoretical values at 95% confidence level, t =2.306, F =6.388

CONCLUSION

The current work describes three validated photometric methods for the determination of MEM in its dosage forms. The drug was derivatized with either NBD-Cl or OPA/NAC reagents in alkaline media. The obtained derivatives were measured spectrophotometrically at 476 and 340 nm, respectively. Moreover, MEM-NBD-Cl reaction product was measured spectro-fluorimetrically in acetone at λex / λem: 455 / 500 nm, offering a sensitive assay allowing the quantitation of low concentrations (0.02 μg/ml) of MEM. The simplicity, selectivity and robustness achieved by these methods advocate their applicability in routine quality control of MEM tablets without interference from commonly encountered tablet excipients.

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