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Research article

Factorial analysis optimization of memantine hydrochloride spectrofluorimetric quantitation via derivatization with o-phthalaldehyde in the absence of thiol

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Key words: Memantine; o-phthalaldehyde; Spectrofluorimetry; Factorial design.

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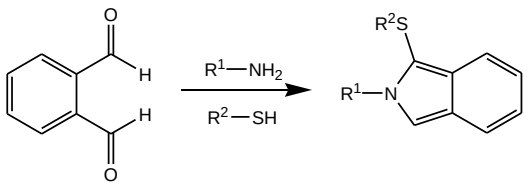
Abstract

Memantine hydrochloride, low-to-moderate affinity noncompetitive N-methyl-D-aspartate receptor antagonist, is used in the treatment of patients with moderate to severe Alzheimer's disease. Chemically, Memantine is an aliphatic tricyclic primary amine compound which lacks any UV or fluorescence properties. Previous studies have reported the spectrophotometric quantification of memantine after derivatization reactions with different reagents. In this paper, we reported the formation of a fluorescent product by reacting memantine hydrochloride and o-phthalaldehyde in the absence of a thiol. Experimental design methodologies were used in the optimization step. The variables under investigation were: pH; the ratio between memantine hydrochloride: o-phthalaldehyde and heating time. The results of statistical analysis of two sequential full factorial design were used for quantification of memantine hydrochloride at optimized reaction condition. Optimal conditions for derivatization were: aqueous NaOH, pH 12.2, memantine hydrochloride:o-phthalaldehyde ratio of 1:5, and heating at 70°C for 45 min. A new eco-friendly spectrofluorimetric derivatization procedure has been developed and validated for analysis of memantine hydrochloride. The linear range was 0.25–10 µg/mL with a quantification limit of 1.97 µg/mL, detection limit of 0.65 µg/mLwith maximum fluorescence intensity development at λex 350 nm and λem 427 nm.

Introduction

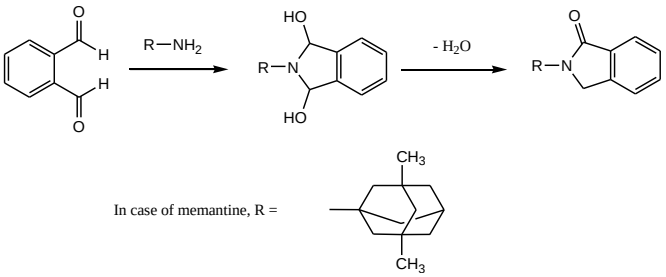
The rapid chemical reaction of o-phthalaldehyde (OPA), a very common flourogenic agent, with primary amines at room temperature in the presence of thiol originate a fluorescent compound, isoindole derivative, which is used for their spectrophotometric analysis [1-3]. In this reaction, schematically shown in Scheme 1, the primary amine and a mercaptan react with OPA under basic condition to form highly fluorescent 1-alkylthio-2-alkyl substituted isoindoles [3, 4].



Scheme1. The general reaction of a primary amine with OPA in the presence of mercaptanto form highly fluorescent 1-alkylthio-2-alkyl substituted isoindoles.

This reaction was used for the spectrophotometric or chromatographic quantification of amino acids [1,5,6] and different drugs having primary amino groups [7-9] after derivatization with OPA/thiol mixture. Different mercaptans were used in literatures such as 2-mercaptoethanol [9, 10], thioglycolic acid [3], 3-mercaptopropionic acid [1, 2], N-acetyl-L-cysteine [11, 12] and other chiral thiols [13]. The influence of different experimental variables, was studied by several authors. [2, 9, 12].

Primary amines [14], amino acids [15, 16] and urea [17, 18] were reported toreact with OPA in the absence of thiol under alkaline condition to yield 1,3-dihydroxyisoindoline derivatives, which upon heating eliminate one molecule of water to form afluorescent product, isoindolin-1-onederivatives, as shown in the following Scheme 2.



Scheme 2. The general reaction of a primary amine with OPA in the absence of thiol to form highly fluorescent isoindolin-1-one derivatives.

Memantine hydrochloride (MTH), low-to-moderate affinity noncompetitive N-methyl-D-aspartate receptor antagonist, is used in treatment of patients with moderate to severe Alzheimer's disease, Parkinsonism, movement disorders [19], dementia syndromes [20], and it has recently been described as a potential treatment for glaucoma [21]. MTH was registered in Europe in 2002 with the indication of moderately severe to severe Alzheimer's disease and was first marketed in the USA in 2003 [22].

Chemically, MTH, 1-amino-3,5-dimethyladamantane hydrochloride, is an aliphatic tricyclic primary amine compound which lacks any UV or fluorescence properties as shown in figure 1 [10, 11]. MTH has a primary amino group and the above-described reactions can be used for its identification and quantification. Previous studies have been reported the spectrophotometric quantification of MTH with the reaction of this compound with OPA and a thiol, [11, 12, 23, 24] but none, to our knowledge, was reported for spectrofluorimetric analysis of MTH after derivatization with OPA in the absence of thiol. There have been some reports about the analysis of the MTH determination by HPLC [25, 26], GC-MS [27, 28], LC-MS [29, 30], and capillary electrophoresis [31]. However, these methods are too much complex, expensive instrumentation, time-consuming sample preparation and long chromatographic elution time for analysis of MTH [10, 25, 26]. Few spectrophotometric methods were developed for the assay of MTH either by UV, colorimety or fluorimetry [11, 32, 33].

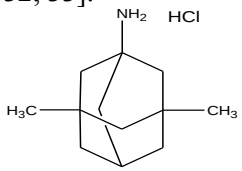


Figure 1. Chemical structure of memantine hydrochloride (MTH), 1-amino-3, 5-dimethyladamant ane hydrochloride.

The aim of this paper was to develop an eco-friendly, simple spectrofluorimetric based analytical method for determination of MTH in bulk and pharmaceutical preparations. Special attention was put on optimization of the reaction of MTH with OPA in the absence of a thiol, forming a fluorescent isoindolin-1-one derivative, to avoid the unpleasant odor and toxicity of free thiols [33]. The optimal conditions of this reaction were systematically optimized by employing full factorial experimental design methodologies [34-36].

Experimental

Chemical

Memexa®Tablets are manufactured by Copad Egypt for trade and pharmaceutical industries (Copad Pharma): Al Obour City-Cairo, Egypt and each tablet claimed to contain 10 mg MTH. MTH (99.9%) was kindly provided by Sigma for Pharmaceutical Industries Co. Mubarak Industrial Zone, 1st Zone; Quesna; Elmenoufia; Egypt. OPA and sodium hydroxide werepurchased from Fluka Chemical Industries, Ltd. (Buchs, Switzerland).Water was obtained by double distillation.

Solutions

Aqueous solutions of sodium hydroxide (pH = 8.5, 10.5 and 12.2) were prepared. Three stock solutions of MTH containing 0.1 mg/mL at three different pH values (pH = 8.5, 10.5 and 12.2) were prepared by dissolving 25 mg of the MTH in (100 mL) NaOH solutions (pH = 8.5, 10.5 and 12.2) and volumes were adjusted to 250 mL with the same solution. Three stock solutions of OPA (2.0 mg/mL) were also prepared by dissolving50 mg OPA in 25mL of NaOH solutions (pH = 8.5, 10.5 and 12.2), and were protected from light and kept at 4°C. In the experimental design optimization, the MTH and OPA standard stock solutions were prepared in NaOH solutions (pH = 8.5, 10.5 and 12.2) to concentrations of 1.0 mg/mL.

Instrumentation and software

Spectrofluorimetric measurements were made inFP- ***6300*** Jasco, (Japan) spectrofluorimeter equipped with a 150 W xenon lamp and connected to personal computer loaded with {Jasco} –{spectra manager} software. The pH measurements were made with HANNA pH 211 Microprocessor, pH Meter with double Junction glass combination electrode. Statistical analysis of data including factorial design was made using Minitab 18®software [37].

General procedures and calibration graphs

Analytical procedure

Aliquots of MTH containing (0.25–20 µg/mL) MTH were transferred to a set of light-protected screw capped test tubes, appropriate volume of OPA was added and mixed well. Test tubes were warmed in a water bath at 70°C then, the contents were allowed to cool. The solutions were transferred to 5.0 mL volumetric flasks and the volume was adjusted to the mark with NaOH solutions. The fluorescence intensity of the resulting solutions was measured at emission 427 nm after excitation at 350 nm. The blank determination without the addition of the analyte was also prepared simultaneously and was used to adjust the background fluorescence intensity. The fluorescence intensity was plotted versus the final drug concentration (µg/mL) to get the calibration graphs and the corresponding regression equations were derived.

Analysis of pharmaceutical preparation

Twenty tablets of Memexa® with labeled amount of 10 mg/tablet was weighed and crushed to fine powder. The powder corresponding to one tablet was weighed,

dissolved in 50 mL aqueous NaOH and sonicated for 10 min. The volume was adjusted to 100mL with NaOH. The solution was filtered and solution (0.5mL) was transferred to 10mL volumetric flask. The analytical procedure was followed and the quantitation was made from the external calibration using the corresponding regression equation.

Result and Discussion

Different authors have described several derivatization reagents used for analysis of MTH, such as FMOC-Cl [5], (2-naphthoxy) acetylchloride, dansyl chloride [38], bromothymol blue [39], sulphonphthalein acid dyes [40] and ninhydrine [34]. In this research, an eco-friendly, simple, one step derivatization reaction for quantification of MTH was achieved by forming a fluorescent product resulting from the reaction of OPA with MTH under alkaline condition which showed maximum absorbance at 427 nm after excitation at 350 nm.

Optimization of derivatization conditions

The general strategy was based on a preliminary evaluation of factors affecting the derivatization reaction between MTH and OPA. The possible interactions between factors will then be investigated by statistical analysis of full factorial experimental design, followed by optimization of the significant factors [34, 36]. Three main factors were appeared to be affecting the derivatization reaction of memantine with OPA; the reaction heating time, pH, and the volume ratio between the MTH and OPA. An initial evaluation of these factors was performed in a univariate way under alkaline condition and it was found that: i) The minimum volume ratio needed for the reaction fluorescence development is (1:1, MTH:OPA). ii) The reaction needs strong alkaline condition to liberate the free amino group as a direct relationship between pH and the fluorescence intensity is reported. iii) Higher absorbance is observed with elevating the temperature and heating time. iv) The formed fluorescent product is stable. (v) Higher fluoresces are observed with NaOH aqueous solutions than with borate or acetate buffers. The results obtained confirmed the formation of stable fluorescent product up to 120 min which has a wavelength of maximum absorbance (427 nm) after excitation at (350 nm).

Optimization of reaction conditions using factorial design

For optimization of derivatization conditions, a multilevel, 2^3 full factorial design was carried out and each experiment was repeated in triplicate using Minitab 18® Software [37]. A set of 24 experiments was performed where two levels for each factor were used as following: heating time (15 and 30 min), pH (5.5 and 10.5), and the ratio of MTH-OPA (0.2 (1:5) and 0.5 (1:5). The reported response for each experiment was the fluorescence intensity of isoindolin-1-one derivative products as reported in Table 1. The effect of each factor was tested using a Student (t) test with a corresponding p-value. All studied factors with p-values less than 0.05 and consequently, are considered statistically significant [36, 41].

Table 1. Full factorial design experiments for optimization of spectrofluorimetric conditions

Run order	Heating time (min)	Ratio (MTH:OPA)	pH	Response
1	15	0.2	8.5	199.38
2	15	0.2	10.5	438.58
3	15	0.5	8.5	104.77
4	15	0.5	10.5	353.70
5	30	0.2	8.5	162.73
6	30	0.2	10.5	570.50
7	30	0.5	8.5	86.64
8	30	0.5	10.5	427.48
9	15	0.2	8.5	191.99
10	15	0.2	10.5	443.34
11	15	0.5	8.5	103.61
12	15	0.5	10.5	357.70
13	30	0.2	8.5	158.93
14	30	0.2	10.5	567.27
15	30	0.5	8.5	83.78
16	30	0.5	10.5	434.79
17	15	0.2	8.5	200.50
18	15	0.2	10.5	446.42
19	15	0.5	8.5	107.37
20	15	0.5	10.5	345.40
21	30	0.2	8.5	160.32
22	30	0.2	10.5	568.10
23	30	0.5	8.5	82.21
24	30	0.5	10.5	422.99

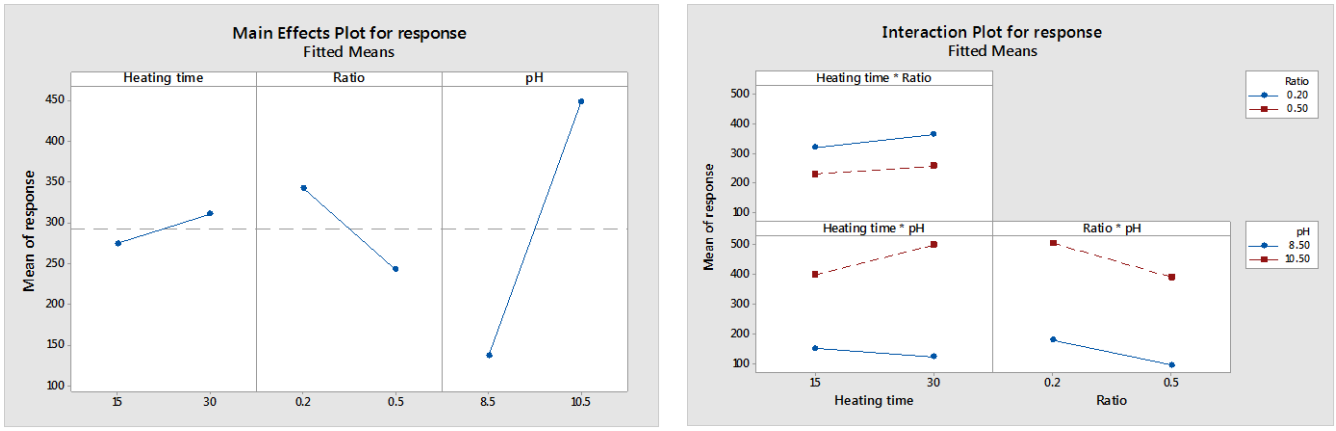
Main effect plots, interaction plots and contour plots shown in figures (2 and 3), revealed that the fluorescence intensity, the response, is optimizing at the maximum level of pH, heating time and minimum level of the ratio between MTH and OPA while an interaction between pH and heating time is also observed. A graphical display of the ordered standardized effect of each factor, Pareto chart (Figure 4 (a)), showed that pH is the most statistically significant factor. Optimum conditions for derivatization reaction was obtained from the response optimizer plot by using the input variable combinations as shown in figure 4 (b). It involves the use of 0.2 (1:5) ratio between (MTH-OPA) at pH 10.5 and heating the reaction mixture at 70^{o}C for 30 min to get the best response.

The optimized spectrofluorimetry conditions were successfully applied to analyze MTH after derivatization with OPA. The linearity of the method was established by

preparing a calibration curve in the range 0.5-20 µg/mL. Triplicates of each experiment were performed and the fluorescence intensity was recorded. The mean fluorescence intensity was plotted against concentration (µg/mL) to construct the calibration curve-1. The correlation coefficient and regression equation were determined as shown in Table 2.

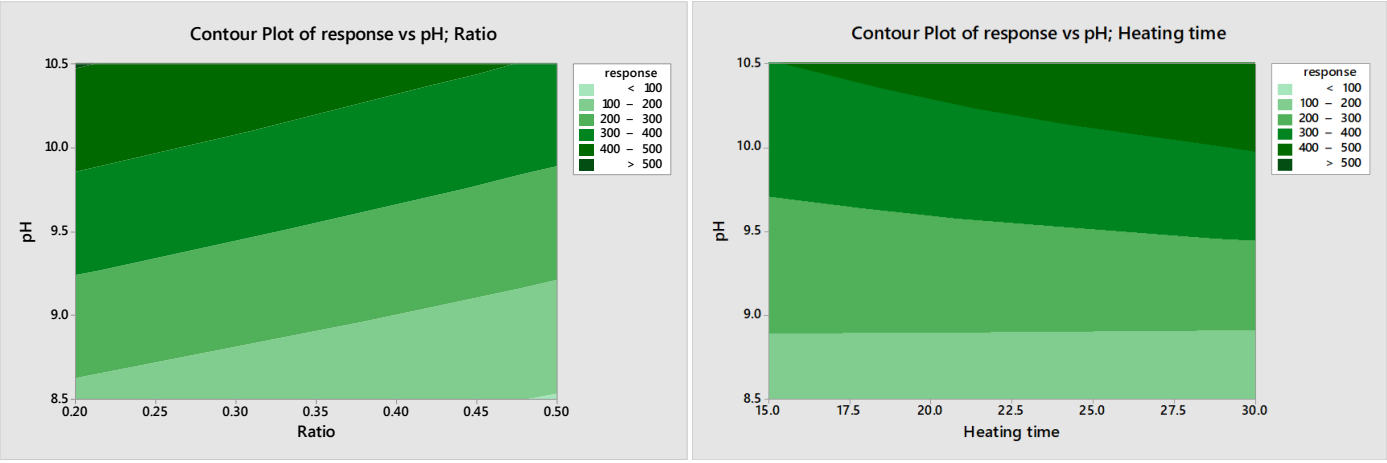
Table 2. Linearity regression data for the calibration plot-1 of MTH

Linearity range	0.5–20 µg/mL
Slope	4.1975
SE of slope	2.542095
Intercept	38.284
SE of Intercept	1.4322
Correlation coefficient (r)	0.9946
SD of Intercept	3.7953
Limit of detection (LOD)	2.9835 µg /mL
Limit of quantitation (LOQ)	9.0410 µg /mL
Equation	y = 4.1975x + 38.284



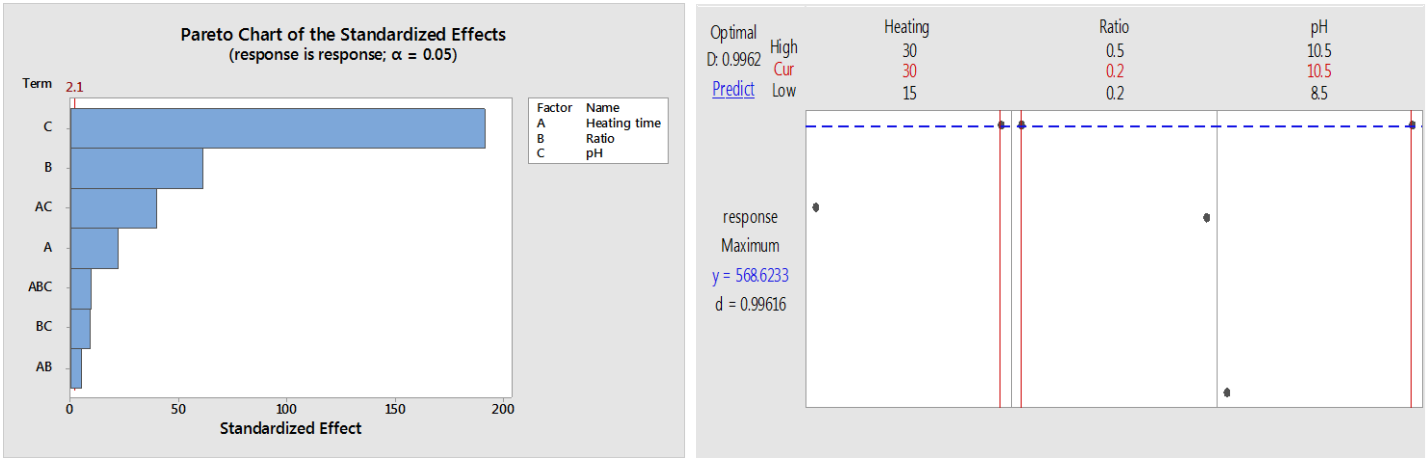
(a) **(b)**

Figure 2. Main effect plot (a) and interaction plot (b) of factors affecting derivatization reaction of MTH with OPA (heating time, MTH-OPA ratio, pH) showing that pH is the most important factor.An interaction between the heating time and pH is also observed.



(a) **(b)**

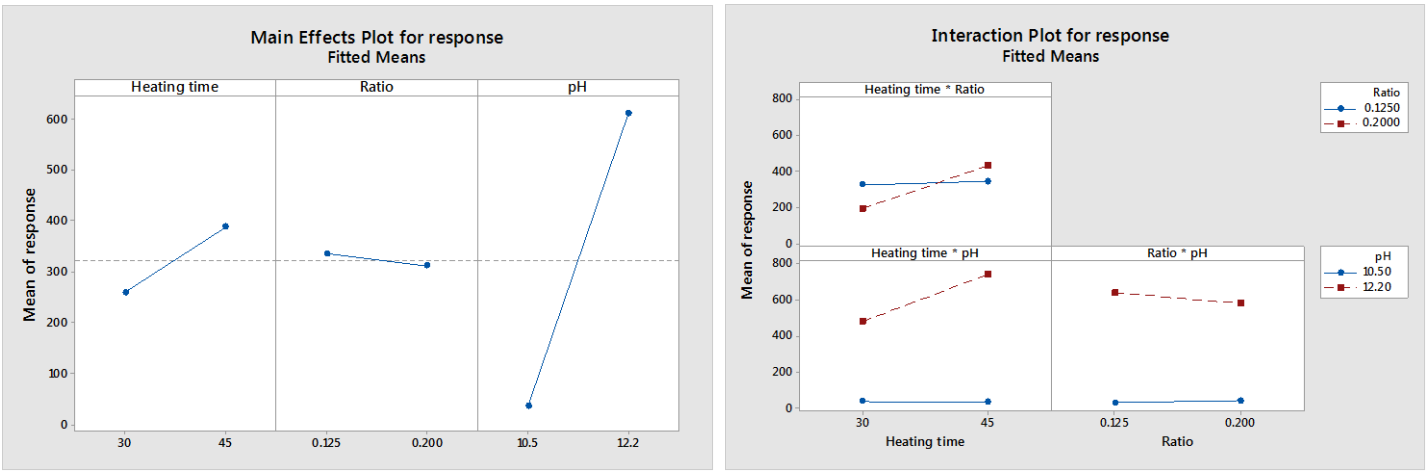
Figure 3. Contour plot (a) of response, fluorescence intensity, versus pH and ratio showed maximum response at high level of pH, 10.5, and low level of MTH-OPA ratio, (0.2, 1:5) while Contour plot (b) of response versus pH and heating time showed maximum response at high level of both pH, 10.5, and heating time, 30 min.



(a) **(b)**

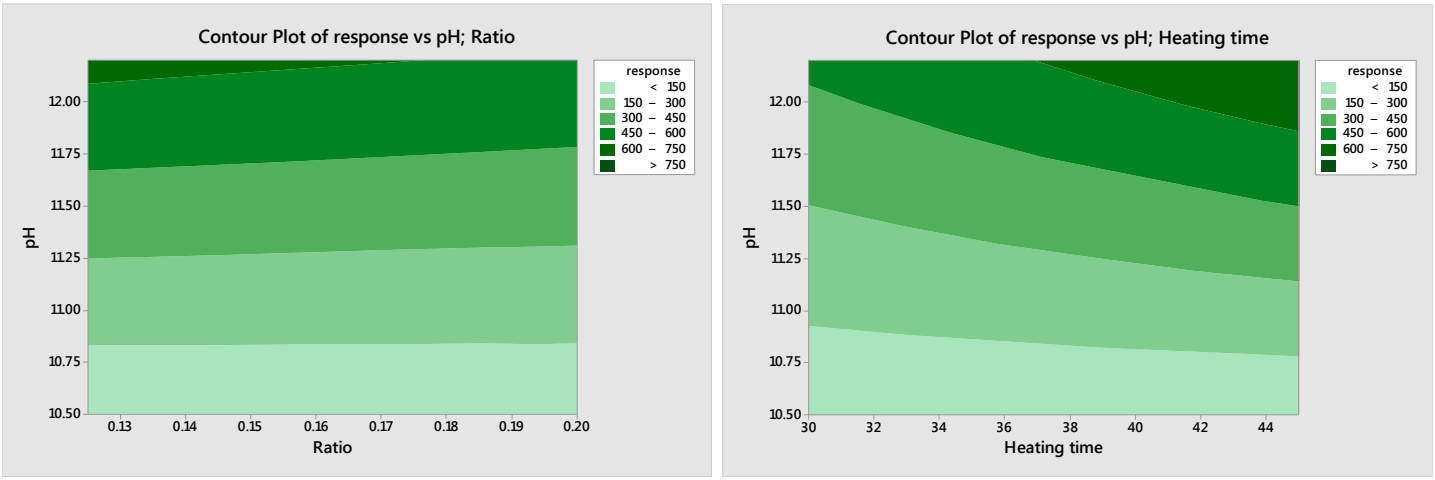
Figure 4. Pareto chart (a) of the standardized effects showed that all studied factors are statistically significant, pH is the most affecting factor, followed by the ratio between MTH-OPA, then heating time while the response optimizer plot (b) indicated the optimum reaction conditions in red color, pH 10.5, ratio 0.2 (1:5) and heating reaction mixture at 70°C for 30 min.

Relying on the results of response optimizer of the aforementioned full factorial design, another factorial design experiment was done to optimize some significant factors with additional levels as following: heating time (30 and 45 min), pH (10.5 and 12.2), the ratio of MTH-OPA (0.125 (1:8) and 0.2 (1:5)) in order to obtain a better reaction conditions and responses. The responses of a set of 24 experiments for a triplicate of another 2^3full factorial design-2arepresented in Table 3. Main effect plots, interaction plots and contour plots shown in Figures (5 and 6), revealed that the fluorescence intensity, the response, is optimizing at the maximum level of pH, heating time and minimum level of the ratio between MTH and OPA while an interaction between heating time and both pH and ratio are observed. Analysis of the minitab 18® statistical results showed that, all factors under study showed p-values less than 0.05 and were considered statistically significant. A graphical display of the ordered standardized effect of each factor, Pareto chart, Figure 7 (a), showed that pH is still the most statistically significant factor. As a result of interaction between the studied factors upon increasing pH and heating time of the derivatization reaction, response optimizer plot, presented in Figure 7 (b), revealed that the optimized reaction condition, higher fluorescence intensity, for the MTH determination is: aqueous NaOH, pH = 12.2; heating at 70°C for 45 min; and the ratio between MTH-OPA = 1:5 (0.2).



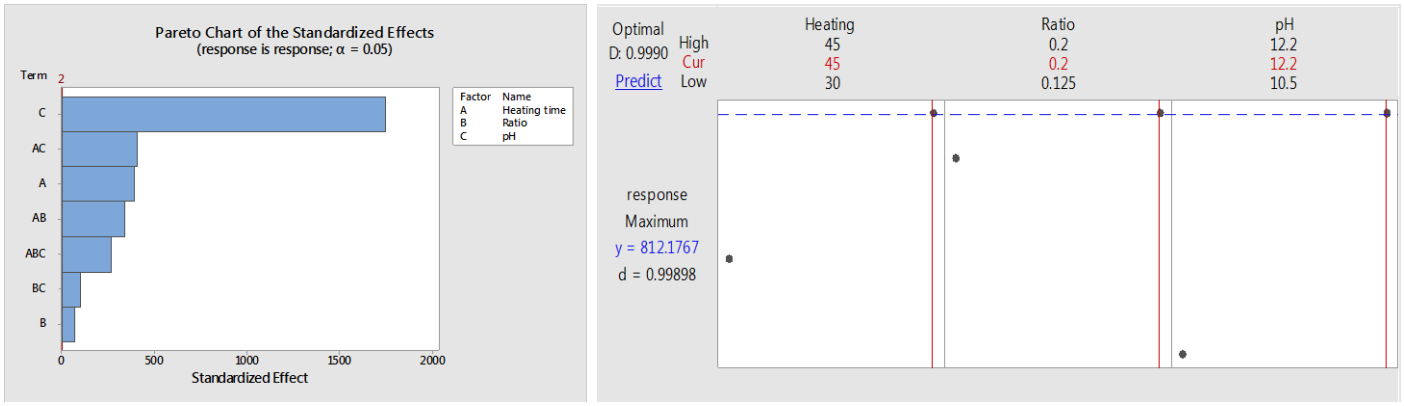
(a) **(b)**

Figure 5. Main effect plot (a)and interaction plot (b) of factors affecting derivatization reaction showing that pH is the most important factor and an interaction between the heating time and pH is observed as well as heating time and the ratio on factorial design-2.



(a) **(b)**

Figure 6. Contour plot (a) of response, fluorescence intensity, versus pH and ratio and contour plot (b) of response versus pH and heating time for factorial design-2. Maximum response is observed at high level of pH, 12.2, low level of MTH-OPA ratio, (0.125, 1:8) and high level of heating time, 45 min.



(a) **(b)**

Figure 7. Pareto chart (a) of the standardized effects of different factors on derivatization experiment for full factorial design-2 showing that all studied factors are statistically significant while the response optimizer plot (b) showed the optimum derivatization conditions in red color, pH 12.2, ratio 0.2 (1:5), and heating time 45 min as a result of interaction between factors.

Final evaluation

A final univariate evaluation was done by increasing, the ratio between MTH-OPA from 0.1 (1:10) to 1.0 (1:1) at pH12.2, and heating the reaction mixture at 70°C for 45 min. The results found are presented in Figure 8. An increase in the fluorescence intensity was observed until a ratio of (1:5) followed by a posterior decrease. With these optimized conditions, the calibration curve was obtained using a series of MTH standard concentrations and the merit of the calibration curve are reported in Table 4. Under the final optimized conditions, the regression data of the calibration curve-2are superior compared to the same calculated data after the first factorial design-1 as observed in Tables 2 and 4. Our developed method showed a comparable better results regarding linearity range (0.25-10 µg/mL), LOD (0.6512 µg/mL), LOQ (1.9735 µg/mL) at (λ_{ex} = 350, λ_{em} = 427 nm) relative to a reported method [11] using OPA in the presence of a thiol which showed the linearity range (5-50 µg/mL), LOD (0.858), and LOQ (2.600) at (λ_{ex} = 340, λ_{em} = 476 nm).A preliminary factorial design-1 of experiment followed by another optimizing factorial design-2 resulted in a more sensitive and accurate procedure approved by the remarkable lower values of the intercept, LOD and LOQ than other reported method using OPA and thiol.

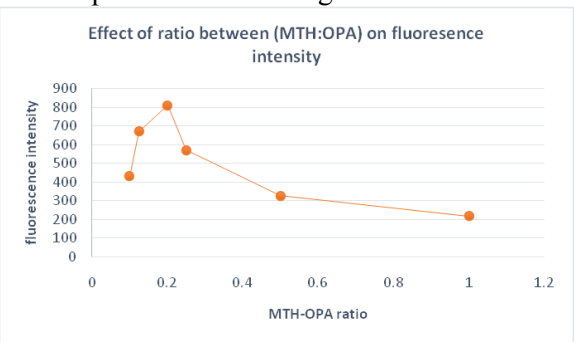


Figure 8. Univariate variation of fluorescence intensity in function of the ratio between MTH-OPA, maximum fluorescence intensity is observed at 0.2 (1:5) ratio.

Table 3. Factorial design experiments-2 for optimization of spectrofluorimetric conditions

Run order	Heating time (min)	Ratio (MTH:OPA)	pH	Response
1	30	0.125	10.5	44.87
2	30	0.125	12.20	607.32
3	30	0.2	10.5	31.04
4	30	0.2	12.20	351.78
5	45	0.125	10.5	16.39
6	45	0.125	12.20	670.20
7	45	0.2	10.5	49.29
8	45	0.2	12.20	811.46
9	30	0.125	10.5	45.25
10	30	0.125	12.20	608.68
11	30	0.2	10.5	32.03
12	30	0.2	12.20	353.02
13	45	0.125	10.5	16.50
14	45	0.125	12.20	669.45
15	45	0.2	10.5	50.44
16	45	0.2	12.20	812.08
17	30	0.125	10.5	45.96
18	30	0.125	12.20	606.99
19	30	0.2	10.5	32.66
20	30	0.2	12.20	350.65
21	45	0.125	10.5	15.89
22	45	0.125	12.20	671.15
23	45	0.2	10.5	50.71
24	45	0.2	12.20	812.99

Table 4. Linearity regression data for the calibration curve-2 of MTH under final optimized condition

Linearity range	0.25–10 µg/mL
Slope	14.152
SE of slope	1.8705
Intercept	24.371
SE of Intercept	1.0538
Correlation coefficient (r)	0.9995
SD of Intercept	2.793
Limit of detection (LOD)	0.6512 µg /mL
Limit of quantitation (LOQ)	1.9735 µg /mL
Equation	y = 14.152x + 24.371

Application of the proposed method to the analysis of MTH in its Tablet dosage form:

The accuracy and precision of the proposed method was assessed by determining the concentration of MTH at three different concentration levels within the linearity range in one day and on three consecutive days. The standard deviation, relative standard deviation, percentage of error and mean recovery obtained by intra-day and inter-day assay were calculated and are summarized in Table 5. The results of the assays are acceptable and reasonable according to ICH guidelines [42].

The specificity of the method was investigated by performing recovery experiments through standard addition technique. For this purpose, a known amount of MTH was added to the pre-analyzed dosage form, Memexa® tablet (10mg), at three different percentage levels (50%, 100% and 150% of that in tablet) and then determined by the proposed methods. The results, in Table 6, showed that no interference from the common dosage form excipients was observed and establishes some degree of specificity of the proposed methods. For the assessment of method robustness, some experimental parameters were interchanged; volume ratio of MTH:OPA (1:5 ±0.1 mL) and pH of NaOH (12.20 ±0.10). The analysis was performed at two different concentrations of MTH (5 and 10µg/mL). The ability remained unaffected by small deliberate variations. The results, presented in Table 7, indicate the acceptable robustness of the proposed methods.

Table 5. Precision and accuracy data forthe determination of MTH of theproposed spectrofluorimetric method

MTH taken (µg/mL)	MTH found (µg/mL) ± SD*	% Recovery	% RSD	% Error
		Intra-day		
5	4.96 ± 0.25	99.20	0.25	0.80
10	9.96 ± 0.82	99.60	0.82	0.40
20	20.06 ± 0.60	100.30	0.60	0.15
		Inter-day		
5	4.95 ± 0.20	99.00	0.20	1.00
10	9.97 ± 0.40	99.70	0.40	0.15
20	19.98 ± 0.15	99.50	0.15	0.05

***The average of three separate determinations.**

Table 6. Results of recovery experiments

Labeled claim (mg)	Pure drug added (mg)	Found ± SD*	% Recovery	% RSD	% Error
10	5	14.87 ± 0.46	99.10	0.46	0.87
10	10	20.05 ± 0.60	100.25	0.60	0.25
10	15	24.94 ± 0.55	99.76	0.55	0.24

***The average of three separate determinations.**

Application of the proposed methods for analysis of MTH in tablet dosage form

The proposed methods were applied to the quantification of MTH in tablet dosage forms purchased from a local pharmacy store. The results, shown in Table 8, suggest that the method is suitable for the determination of MTH with good accuracy and precision. The excipients in the dosage forms do not interfere in the assay procedure and three replicates were determined.

Table 7. Evaluation of the robustness of the proposed spectrofluorimetric method using OPA for the determination of MTH

Parameter	MTH taken (µg/mL)	MTH found (µg/mL) ± SD*	Average % Recovery	% RSD	% Error
Volume ratio of MTH:OPA (1:5 ± 0.1 mL)	5	4.95	99.00 ± 0.53	0.53	0.50
pH of NaOH (12.20 ± 0.1)	5	4.90	98.00 ± 0.65	0.66	1.30
Volume ratio of MTH:OPA (1:5 ± 0.1 mL)	10	10.06	100.60 ± 0.75	0.75	0.20
pH of NaOH (12.20 ± 0.1)	10	9.87	98.70 ± 0.38	0.38	0.93

***The average of three separate determinations.**

Table 8. Assay results for the quantification of MTH in tablet dosage form using the proposed spectrofluorimetric method using OPA

Formulation	Amount taken (µg)	Amount Found ± SD[b]	% Recovery	% RSD
Memexa® tablet (10mg MTH/tablet) [a]	2	2.05 ± 0.95	102.50	0.95
	5	4.98 ± 0.80	99.60	0.81
	10	9.95 ± 0.12	99.50	0.12

[a]Copad Pharma, Egypt for trade and pharmaceutical industries: Cairo, Egypt
[b]The average of three separate determinations.

Conclusion

An eco-friendly, new spectrofluorimetric method was developed for the quantification of MTH using OPA in the absence of thiol. Two sequential factorial design experiments were developed to optimize the factors affecting the derivatization reaction between MTH and OPA. The developed method is validated as per ICH guidelines. It was observed that all validation parameters such as linearity, precision, accuracy, selectivity, and robustness convene the predetermined acceptance criteria. Thus, it has been concluded that the proposed method is validated for the routine analysis of the drug in pure and tablet dosage form.The proposed spectrofluorimetric method has the advantages of simplicity, precision, accuracy and convenience for the quantitation of MTH. Consequently, the proposed method can be used for the quality control of the cited drug and can be extended for their routine analysis in dosage forms.

References

1. Fiorino A, Frigo G and Cucchetti E: Liquid chromatographic analysis of amino and imino acids in protein hydrolysates by post-column derivatization with o-phthalaldehyde and 3-mercaptoproptonic acid. Journal of Chromatography 1989; 416: 83-92.
2. Rao N, Maurya PK, Shinde DD and Khalid S: Precolumnderivatization followed by liquid chromatographic separation and determination of tramiprosate in rat plasma by fluorescence detector: Application to pharmacokinetics. Journal of Pharmaceutical and Biomedical Analysis 2011; 55: 282–287.
3. Srikanth R, Reddy DR and Venkatesan CS: Development and validation of rp-HPLC pre-column derivatisation for the trace level determination of tert. Butylamine in tigecycline drug substance. International Journal of Pharmaceutical and Biological Science 2013; 4 (2): 522-531.
4. Yan CC and Huxtable RJ: Fluorimetric determination of monobromobimane and o-phthalaldehyde adducts of y-glutamylcysteine and glutathione: application to assay of y-glutamylcysteinylsynthetase activity and glutathione concentration in liver. Journal of Chromatography B1995; 672: 217-224.
5. Herbert P, Barros P, Ratola N and Alves A: HPLC determination of amino acids in musts and port wine using OPA/FMOC derivatives.Journal ofFoodScience2000; 65: 1130-1133.
6. Frattinia V and Lionettib C: Histamine and histidine determination in tuna fish samples using high-performance liquid chromatography derivatization with o-phthalaldehyde and fluorescence detection or UV detection of ''free'' species. Journal of Chromatography A1998; 809: 241–245.
7. Musson DG, Maglietto SM and Bayne WF: determination of the antibiotic fludalanine in plasma and urine by high-performance liquid chromatography using a packed-bed, post-column reactor with o-phthalaldehyde and 2-mercaptoethanol.Journal of Chromatography 1985; 338: 357-367.
8. Navas MJ and Jimenez AM: Analytical methods to determine anti-influenza drugs. Critical Reviews in Analytical Chemistry 2011; 41: 81–97.
9. Al Deeb SK, Hamdan II and Al Najjar S M: Analytical methods to determine anti-influenza drugs. Talanta 2004; 64: 695–702.
10. Dousa M, Pivonkova V and Sykora D: Optimization of o-phtaldialdehyde/2-mercaptoethanolpostcolumn reaction for the hydrophilic interaction liquid chromatography determination of memantine utilizing a silica hydride stationary phase. Journal of Separation Science 2016; 39: 3145–3155.
11. Michail M, Daabees H, Beltagy Y, Abdelkhalek M and Khamis M: Spectrophotometric and spectrofluorimetric determination of memantine hydrochloride in bulk and pharmaceutical preparations. International Journal of Pharmacy and Pharmaceutical science 2011; 3: 180-185.
12. Zhao M and Bada JL: Determination of α-dialkylamino acids and their enantiomers ingeological samples by high-performance liquid chromatography after derivatizationwith a chiral adduct of o-phthalaldehyde. Journal of Chromatography A 1995; 690: 55-63.
13. Desai DM and Gal J: Enantiospecific drug analysis via the ortho-phthalaldehyde/homochiral thiol derivatization method. Journal of Chromatography 1993; 629: 215-228.
14. DoMinh T, Johnson AL, Jones J E and Senise PP: Reactions of phthalaldehyde with ammonia and amines. Journal of Organic Chemistry 1977;42:4217-4221.
15. Forras KG, Leitner A, Akasaka K and Lindner W: Comparative study on the use of ortho- phthalaldehyde, naphthalene-2,3-dicarboxaldehyde and anthracene-2,3-dicarboxaldehyde reagents for α-amino acids followed by the enantiomer separation of the formed isoindolin-1-one derivatives using quinine-type chiral stationary phases. Journal ofChromatography A 2005; 1083: 80–88.
16. Alvarez-Coque MCG, Hernández MJM, Camañas RMV and Fernández CM: Spectrophotometric determination of cysteine with o-phthalaldehyde in the absence of thiol. analytical letters 1988; 21(9): 1545-1559.

17. Maliha B, Hussain I, Tariq MI and Siddiqui HL: Mechanistic studies on the reaction of o-phthalaldehyde (OPTA) with urea and its N-alkyvaryl derivatives. Journal of The Chemical Society of Pakistan 2009; 31: 829-837.
18. Jung D, Biggs H, Erikson J and Ledyard PU: New colorimetric reaction for end-point, continuous-flow, and kinetic measurement of urea. Clinical Chemistry 1975; 21: 1136-1140.
19. Schneider E, Fischer PA, Clemens R, Balzereit F, Funfgeld EW, Haase HJ: Effects of oral memantine administration on parkinson symptoms. Results of a placebo-controlled multicenter study. Deutsche MedizinischeWochenschrift 1984; 109: 987-990.
20. Hanney M, Prasher V, Williams N, Jones EL, Aarsland D, Corbett A, Lawrence D, Yu Land Tyrer S: Memantine for dementia in adults older than 40 years with Down's syndrome (MEADOWS): a randomised, double-blind, placebo-controlled trial. Lancet 2012; 379: 528–536.
21. Yu¨cel YH, Gupta N, Zhang Q, Mizisin AP, Kalichman MW and Weinreb RN: Memantine protects neurons from shrinkage in the lateral geniculate nucleusin experimental glaucoma. Archives of Ophthalmology 2006; 124: 217-225.
22. Winblad B, Jones RW, Wirth Y, Stofler A and Mobius HJ: Memantine in moderate to severe alzheimer's disease: a meta-analysis of randomised clinical trials. Dementia and. Geriatric Cognitive Disorders 2007; 24: 20–27.
23. Suckow RF, Zhang MF, Collins ED, Fischman MW and Cooper TB: Sensitive and selective liquid chromatographic assay of memantine in plasma with fluorescence detection after pre-column derivatization. Journal of Chromatography B 1999; 729: 217–224.
24. Zarghi A, Shafaati A, Foroutan SM, Khoddam A and Madadian B: Sensitive and rapid HPLC method for determination of memantine in human plasma using opaderivatization and fluorescence detection: application to pharmacokinetic studies. ScietiaPharmaceutica 2010; 78: 847–856.
25. Puente B, Hernandez E, Perez S, Pablo L, Prieto E, Garcia MA and Bregante MA: Determination of memantine in plasma and vitreous humour by HPLC with precolumnderivatization and fluorescence detection. Journal of Chromatographic Science 2011; 49: 745-752.
26. Jing S-J, Li Q-L and Jiang Y: A new simultaneous derivatization and microextration method for the determination of memantine hydrochloride in human plasma. Journal of Chromatography B 2016; 1008: 26-31.
27. Leis HJ and Fauler G: Quantitative analysis of memantine in human plasma by gas chromatography/negative ion chemical ionization/mass spectrometry. Journal of Mass Spectrometry 2002; 37: 477–480.
28. Kornhuber J, Kennepohl EM, Bleich S, Wiltfang J, Krau T, Reulbach U, Meineke I: Memantine pharmacotherapy: a naturalistic study using a population pharmacokinetic approach. Clinical Pharmacokinetic 2007; 46: 599–612.
29. César IC, Byrro RMD, Silva FFS, Mundim IM, Teixeira LS, Crunivel de Abreu F and Pianetti GA: Bioequivalence study of two oral formulations of memantine tablets in healthy brazilian volunteers after a single dose administration. Journal of Bioequivalence and Availability 2012; 4: 14-17.
30. Bhateria M, Ramakrishna R, Pakala DB and Bhatta RS: Development of an LC–MS/MS method for simultaneous determination of memantine and donepezil in rat plasma and its application to pharmacokinetic study.Journal Chromatography B 2015; 1001: 131-139.
31. Yeh HH, Yang YH and Chen SH: Simultaneous determination of memantine and amantadine in human plasma as fluorescein derivatives by micellar electrokinetic chromatography with laser-induced fluorescence detection and its clinical application. Electrophoresis 2010; 31: 1903-1911.
32. Jagathi V, Anupama B, Praven PS and Rao GD: Spectrophotometric determination of memantine in bulk and pharmaceutical formulations. International journal of Current Pharmaceutical Research 2010; 2: 17-18.
33. Munday R: Toxicity of thiols and disulphides: involvement of free-radical species.Free Radical Biology and Medicine 1989; 7(6): 659-673.
34. Omara HA and Younis HM: Spectrophotometric determination of alzheimer's drug, memantine hydrochloride in biological samples using ninhydrin and ferric chloride. IOSR Journal of Applied Chemistry 2017; 10: 52-59.
35. Leitao JMM and Esteves da Silva JCG: Factorial analysis optimization of a diltiazem kinetic spectrophotometric quantification method. AnalyticaChimicaActa 2008; 609: 1–12.
36. Abdel-Aziz O, Ayad MF and Tadros MM: Compatible validated spectrofluorimetric and spectrophotometric methods for determination of vildagliptin and saxagliptin by factorial design experiments. SpectrochimicaActa Part A: Molecular and Biomolecular Spectroscopy 2015; 140: 229–240.
37. Minitab 18 Statistical Software (2017). [Computer software]. State College, PA: Minitab, Inc. (www.minitab.com).
38. Duh TH, Wu HL, Pan CW and Kou HS: Fluorimetric liquid chromatographic analysis of amantadine in urine and pharmaceutical formulation. Journal of Chromatography A 2005; 1088:175–181.
39. Rani AP, Bhawani S, Nagalakshmi C and Sekaran CB: Determination of memantine hydrochloride by spectrophotometry using anionic dyes, bromothymol blue and solochrome black T, in bulk and tablet dosage forms. Chemical Science Journal 2012; 60: 1-9.
40. Omara HA and Amin AS: Extractive-spectrophotometric methods for determination of alzheimer's drug in pharmaceutical formulations and in biological samples using sulphonphthalein acid dyes. Canadian Chemical Transactions 2016; 4: 17-31.
41. Kiendrebeogo M, Choisnard L, Lamien CE, Meda A, Wouessidjewe D andNacoulmaOG: Experimental design optimization for screening relevant free phenolic acids from various preparations used in Burkina Faso folk medicine. African Journal of Traditional, Complementary and Alternative Medicines 2006; 3: 115-128.
42. ICH-Q2B Validation of Analytical Procedures: Methodology International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, Geneva, Switzerland 1996.