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A spectrophotometric assay for monoamine oxidase activity with 2, 4-dinitrophenylhydrazine as a derivatized reagent

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ABSTRACT

A simple, rapid and reliable spectrophotometry was developed to determine monoamine oxidase (MAO). In this study, 2,4-dinitrophenylhydrazine (DNPH), a classic derivatizing reagent, was used to detect MAO-dependent aldehyde production; and traditional DNPH spectrophotometry was simplified. Benzylamine and serotonin oxidation were catalyzed by MAO-B and MAO-A, respectively, to aldehydes. These were derivatized with DNPH, and the corresponding quinones were further formed by adding NaOH. These DNPH derivatives with large conjugated structures were directly measured spectrophotometrically at 465 nm and 425 nm, without the need for precipitating, washing and suspending procedures. The addition of NaOH caused a red shift of the maximum absorption wavelength of these derivatives, which reduced the interference of free DNPH. MAO-B protein was as low as 47.5 μg in rat liver with correlation coefficients ranging within 0.995–0.999. This method is 2–3 times more sensitive than direct spectrophotometry. The detection of MAO inhibition through this method showed that IC_{50} values of rasagiline are 8.00×10^{-9} M for MAO-B and 2.59×10^{-7} M for MAO-A. These results are similar to the values obtained by direct spectrophotometry. Our study suggests that DNPH spectrophotometry is suitable to detect MAO activity, and has the potential for MAO inhibitor screening in the treatment of MAO-mediated diseases.

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1. Introduction

Monoamine oxidase (MAO) [1,2] (MAO; EC 1.4.3.4), a flavoenzyme of the mitochondrial outer membrane, plays an important role in the regulation of the metabolism of exogenous amines, the level of neurotransmitters (such as dopamine, norepinephrine and serotonin) and intracellular amine stores. MAO exists as two isoforms (MAO-A and MAO-B), according to their substrate specificity and sensitivity to specific inhibitors [3]. MAO-A preferentially oxidizes norepinephrine (NE) and serotonin (5-hydroxytryptamine, 5-HT). In contrast, MAO-B mainly oxidizes phenethylamine (PEA) and benzylamine [4]. MAO-A and MAO-B are widely expressed in most tissues at different concentrations, as shown in Table 1 [1]; and abnormal MAO activities are linked to various diseases [5,6]. Since amine neurotransmitters are metabolized extensively by MAO in the CNS system [7], an increase in MAO expression or activity may be related to depression, Parkinson's disease and Alzheimer's disease. At present, selective MAO-A and MAO-B inhibitors have been used to treat the aforementioned and other diseases [6,8]. Thus the exploration of proper methods to

Abbreviations: 5-HT, 5-hydroxytryptamine; DNPH, 2, 4-dinitrophenylhydrazine; MAO, monoamine oxidase; MAO-A, monoamine oxidase-A; MAO-B, monoamine oxidase-B; MAOI, monoamine oxidase inhibitor.

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Table 1
Distribution of MAO-A and MAO-B in humans and in the brains of selected species.

Tissue	Total activity (%)	
	MAO-A	MAO-B
Brain		
Human (striatum:all brain regions) and Guinea pig	20	80
Monkey and Cat	25	75
Pig	40	60
Rat	55	45
Mice, Glia and astrocytes	50	50
Aminergic neurons	100	0
Other tissues		
Liver(human, rat, rabbit) and Lings (rat)	55	45
Small intestine	~75	25
Kidney (rat)	25	75
Human platelet and Chromaffin cell	5	95
Pheochromocytoma and PC-12 cells	95	5

determine MAO activity is crucial for disease diagnosis, and in screening more potent MAO inhibitors.

MAO activity has been determined by measurement of oxygen consumption and the concentration of hydrogen peroxide (H_2O_2) or an oxidized monoamine product based on their catalytic oxidative deamination of monoamines [9]:

$$RCH_2NR_1R_2 + H_2O + O_2 \rightarrow RCHO + NR_1R_2 + H_2O_2$$

The measurement of oxygen consumption by an oxygen-sensitive electrode requires specialized equipment, and is not appropriate for the rapid measurement of a large numbers of samples [10]. The H_2O_2 method is insensitive and not specific for MAO, because most biological and synthetic compounds also absorb at 230 nm. Additionally, complicated cellular processes could influence the concentration of H_2O_2 [10,11]. Aldehydes, which are oxidized monoamine products, are produced by the incubation of amine substrates with MAO; and these can be detected by spectrophotometry. This method is widely used without requiring expensive or specialized equipment and reagents. However, this method is limited for the measurement of MAO with low activity or concentration. Thus, improving the sensitivity of the spectrophotometric assay is essential to detect MAO activity, and this has the potential to screen MAO inhibitors in MAO-mediated disease treatment.

It has been reported that carbonyls can be measured through the direct or indirect reaction of carbonyl moieties with reagents [12] such as DNPH, tritiated sodium borohydride, fluorescein thiosemicarbazide and fluorescein amine, since these derivatized compounds possess the characteristic of absorbance, radioactivity, or fluorescence, respectively. DNPH has been widely used to determine carbonyl groups at approximately 370 nm [14]. However, the use of traditional DNPH spectrophotometry is time-consuming and labor intensive due to complicated process, including precipitating, washing and re-suspending with organic solvents (ethanol/ethyl acetate and benzene). Moreover, free DNPH also has absorption at approximately 370 nm; hence, the extraction and washing steps of these derivatives are necessary to remove the interference of unreacted DNPH.

Mesquita et al. reported a simplified DNPH spectrophotometric assay to quantitatively determine carbonyls. The key procedure is that the addition of NaOH makes the reaction system alkalizate after adding DNPH [15]. This simplified method reduced the interference of DNPH by shifting the maximum absorbance wavelength of the derivatives. This allowed for the direct measurement in the sample solution without the aforementioned process described in the traditional DNPH method.

In order to improve the sensitivity of spectrophotometry, DNPH was used to measure MAO activity in this study; and MAO-dependent aldehyde products were derivatized to form DNPH derivatives with large conjugated structures and strong UV absorption. In addition, we modified the traditional DNPH spectrophotometry, simplified the procedure, and reduced interference from free DNPH, as reported by Mesquita et al. A reaction scheme (Fig. 1) illustrated the mechanism of this proposed method. The sensitivity of this proposed method for the measurement of MAO activity was compared with direct spectrophotometry, and the validation of the proposed method was carried out by determining MAO inhibition by monoamine oxidase inhibitors (MAOI).

2. Material and methods

2.1. Materials

MAO-B inhibitor (rasagiline, [N-propargyl-1R(+)-aminoindan]), and 5-HT creatinine sulfate monohydrate (1064561, TCL) were used in this study. Benzylamine, DNPH and Triton X-100 were purchased from Adamas Reagent Co., Ltd. All other reagents were of analytical grade.

2.2. MAO sample preparation

Male Sprague-Dawley rats (13–15 months old) were obtained from the Experimental Animal Center of Chongqing Medical University. These rats were maintained on a 12-h light–dark cycle, and fed standard rat chow and tap water ad libitum. Rats were sacrificed by decapitation, and livers and brains tissues were dissected out, washed with sucrose (0.3 M) and frozen at −80 °C until analyzed. Rat liver mitochondrial MAO was isolated by the method reported by Castillo [16] with a slight modification. The liver tissue (5.0 g) or brain tissue (2.5 g) was homogenized at 1:40 (w/v) in 0.3 M of ice-cold sucrose, and centrifuged at 1824 g for 10 min. The supernatants were collected and further centrifuged at 12,768 g for 35 min to obtain crude MAO protein precipitations. These precipitates were resuspended in 500 μl (for liver) or 250 μl (for brain) of 0.3 M of sucrose, and were layered onto 40 ml (for liver) or 20 ml (for brain) of 1.2 M of sucrose. The precipitates were centrifuged in 1.2 M of sucrose at 12,687 g for 40 min, in order to obtain MAO protein precipitates. Following a single wash in potassium phosphate buffer (pH 7.60, 100 mM), the pure liver and brain MAO protein precipitates were suspended in 40 ml (for liver) and 10 ml (for brain) of potassium phosphate buffer; and stored in aliquots of 1 ml at −80 °C for subsequent analysis. All steps were performed at 0–4 °C conditions.

The protein concentrations of MAO protein precipitates were determined using the Bicinchoninic acid (BCA) method [17], with bovine serum albumin (BSA) as the standard. MAO protein concentration was expressed as microgram per microliter (μg/μl).

3. Assay procedure

3.1. DNPH spectrophotometry

The DNPH spectrophotometry reported by Basha [18] was modified to detect MAO activity. The assay mixtures (1.5 ml) contained potassium phosphate buffer (pH 7.60, 25 mM), 200 μl of MAO protein homogenates and benzylamine (for detecting MAO-B), or 5-HT (for detecting MAO-A). The solution was incubated for 20 min at 37 °C before adding 200 μl of 0.016 M benzylamine in buffer or 150 μl of 0.02 M 5-HT and the incubation was continued for 60 min. Next, 400 μl of 2 M DNPH in 1 M HCl was added. After incubation for 40 min at room temperature, 2 ml of 1.25 M NaOH containing 5 g/l of Triton X-100 was added; and the reaction

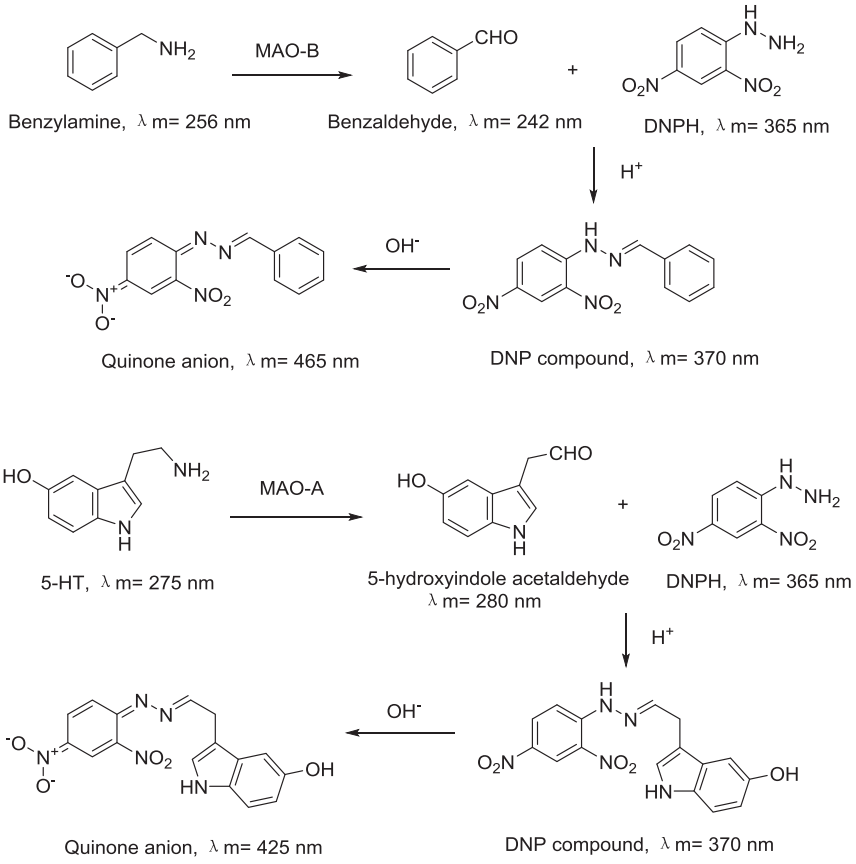

Fig. 1. The reaction scheme for catalytic oxidative deamination of monoamines and DNPH derivatives of target aldehydes (benzaldehyde or 5-hydroxyindole acetaldehyde).

mixture was kept for an additional 30 min at room temperature. Finally, absorption was measured by a spectrophotometer at 465 nm and 425 nm for MAO-B and MAO-A, respectively.

3.2. Direct spectrophotometry

The activities of MAO-A and MAO-B were determined by UV–Vis spectrophotometry. Briefly, 800 μl of a solution containing potassium phosphate buffer (pH 7.60, 100 mM), 200 μl of MAO protein homogenates and benzylamine (for detecting MAO-B) or 5-HT (for detecting MAO-A) was incubated for 20 min at 37 °C. The reaction was started by adding 150 μl of 0.016 M benzylamine in 100 mM buffer or 200 μl of 0.02 M 5-HT, and incubated for 60 min. The reaction was stopped by adding 200 μl of 10% perchloric acid or 200 μl of 2 M HCl. Then, 3 ml of hexamethylene (for detecting MAO-B) or n-butyl acetate (for detecting MAO-A) was added to the reaction solution; and the tubes were vortexed for 3 min. Following centrifugation at 10,625 g for 6 min, absorbance was determined using a spectrophotometer at 242 nm and 280 nm for MAO-B and MAO-A, respectively.

3.3. Determination of MAO inhibitory activity by MAO inhibitors in vitro

The stock solution of rasagiline was prepared with ultra-pure water, and the preincubation was carried out in the presence of increasing concentrations of rasagiline. In order to compare this assay with direct spectrophotometry, MAO-B and -A activities were determined by DNPH spectrophotometry and direct spectrophotometry, as described above. Finally, IC_{50} values of rasagiline for MAO-B and MAO-A were calculated using the Statistical Product and Service Solutions (SPSS) program.

4. Results and discussion

4.1. UV spectroscopic characterization

The absorption spectra of reaction substrates and products were measured at room temperature using a spectrometer of 1 nm spectral resolution from 800 nm to 200 nm. The concentrations of benzylamine, 5-HT and DNPH were 0.0008 M, 0.0002 M and 0.0125 M, respectively; and the absorption spectra are shown in Fig. 2. The results indicate that the absorption peak of DNPH-derivatives for MAO-B and MAO-A are at 465 nm and 425 nm, respectively, without interference from 5-HT (275 nm), benzylamine (256 nm) and DNPH (365 nm). The alkalized reaction system with the addition of NaOH and the transformation from DNPHydrazones to quinone anion not only simplifies the processes of protein precipitating, washing and redissolving, but also avoids the use of organic solvents such as benzene, ethanol/ethyl acetate, and guanidine hydrochloride. Compared to the method reported by Basha [18], our method shortened experimental time from approximately 200 min–100 min. Moreover, the alkaline condition shifted the maximum wavelength of the DNPH derivatives from 375 nm [19] to 465 nm (for determining MAO-B) and 425 nm (for determining MAO-A), in which interference from the absorption of free DNPH or other substances were modest.

4.2. Optimization of DNPH spectrophotometry

In order to improve the sensitivity further, experimental conditions containing the concentration of substrates, pH and the

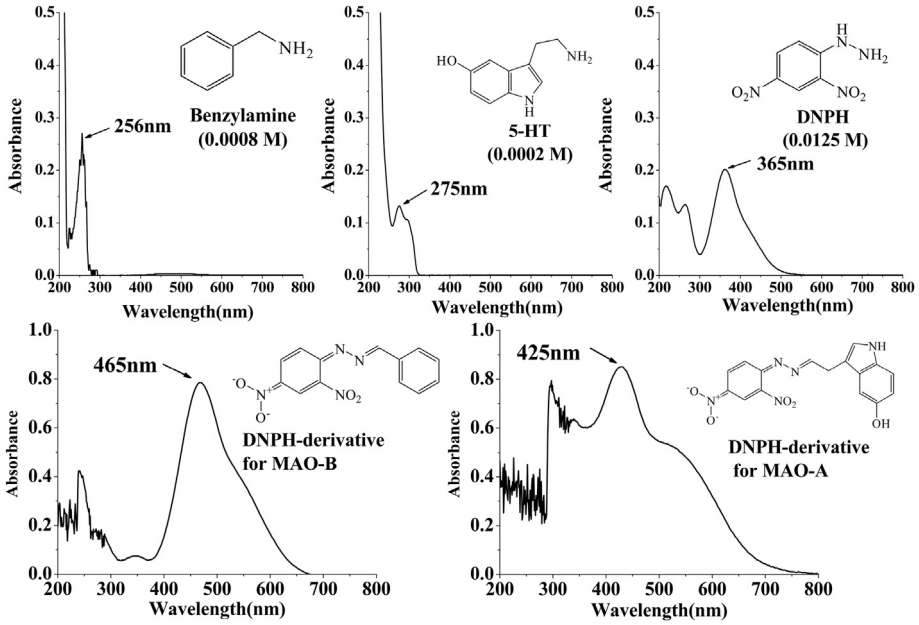

Fig. 2. Absorption spectra (800 nm–200 nm) of 5-HT (0.0002 M), benzylamine (0.0008 M), DNPH (0.0125 M), DNPH-derivatives for MAO-B and MAO-A, and the MAO protein were 234.3 and 468.5 μg, respectively, to obtain DNPH-derivatives for MAO-B and MAO-A. The graph shows that the absorption peaks are at 275 nm, 256 nm, 365 nm, 465 nm and 425 nm, respectively.

concentration of potassium phosphate buffer, as well as reaction time and DNPH derivatizing time were optimized using four liver MAO protein homogenates (4.69 μg/μl) for each experimental condition. The effects of pH and the concentration of the potassium phosphate buffer were evaluated in various concentrations (25, 50, 75 and 100 mM) and pHs (7.20, 7.40, 7.60 and 7.80). These results indicated that the increase in potassium phosphate buffer concentration was associated with a significant reduction in MAO-B activity, but this had minor effects on MAO-A activity. Furthermore, these maximum absorbances for MAO-A and MAO-B were obtained with the potassium phosphate buffer (pH 7.60, 25 mM) (Fig. 3a and b). In order to measure the substrate content required to react with MAO proteins, the influence of substrate concentration was investigated between 0.8 and 4 mM. It was found that benzylamine (3.2 mM) and 5-HT (4 mM) produced the maximum absorbance (Fig. 3c). In order to determine the time required for the amine substrate (benzylamine or 5-HT) to react with the MAOs, the influence of reaction time was also investigated. The results indicated that the increase in time was associated with a corresponding increase in absorbance up to 60 min, as shown in Fig. 3d. Furthermore, in order to determine the time required for DNPH to react with all produced aldehydes, the influence of DNPH derivatizing time was investigated. Maximum absorbance was obtained after for 40 min of DNPH derivatization. Thus, 40 min was used as the optimum DNPH derivatizing time (Fig. 3e).

4.3. Validation of DNPH spectrophotometry

The validity of the proposed method was evaluated following Bio-analytical Method Validation. MAO protein concentration was measured by BCA assay, and average total protein concentrations of the liver and brain MAO protein precipitations were found to be approximately 4.69 and 3.00 μg/μl, respectively. The calibration curves were constructed in rat liver MAO proteins under optimum conditions. Absorbance for various concentrations of MAO (from 23.4 to 468.5 μg) was determined by DNPH spectrophotometry and compared to direct spectrophotometry. The absorption spectra (Fig. 4) and calibration curves (Fig. 5) were constructed using the origin 8 program. The spectra showed that the absorption of derivatized compounds increased with the increase in MAO protein concentration. Fig. 5 shows a linear relationship between MAO protein concentrations and absorbance with correlation coefficients ranging from 0.995 to 0.999. Regression equations for MAO-B and MAO-A were $A = 0.00421x$ and $A = 0.00258x$ by DNPH spectrophometry, respectively; and $A = 0.00151x$ and $A = 0.00074x$ by direct spectrophotometry, respectively.

There is an excellent linear relationship between DNPH spectrophotometry and direct spectrophotometry for the measurement of MAO activity, whereas the slopes of DNPH spectrophotometry were greater than that obtained by direct spectrophotometry based on regression equations. This indicates that the DNPH spectrophotometric assay is more sensitive than is direct spectrophotometry. Compared to direct spectrophotometry, less MAO protein is needed when using the DNPH spectrophotometric assay to attain equivalent absorbance. Based on regression equations, the sensitivity of DNPH spectrophotometry was found to be approximately 2.8 and 3.5-fold greater than that obtained with the direct spectrophotometric method for measuring MAO-B and MAO-A activities, respectively. The possible reason could be that DNPH derivatives for MAO-A and MAO-B possess different conjugated structures, and usually have different UV absorption coefficients; which might lead to difference in sensitivity.

Sensitivity and precision were determined by measuring the activity of equal amounts of MAO proteins (4.69 μg/μl in the liver, 3.00 μg/μl in the brain) from rat liver and brain. In DNPH spectrophotometry, it was shown that the relative standard deviation (RSD) values for precision (n = 5) and repetition (n = 3) were 0.69–3.34% and 3.92–7.44%, respectively. In direct spectrophotometry, it was found that the RSD values for precision (n = 5) and repetition (n = 3) were 2.54–3.29% and 1.26–8.09%, respectively. Table 2 summarizes the results for precision and repetition, and indicates that both DNPH spectrophotometry and direct spectrophotometry povide good precision and repetition in the measurement of MAO activity. However, Fig. 6 and Table 2 show that the

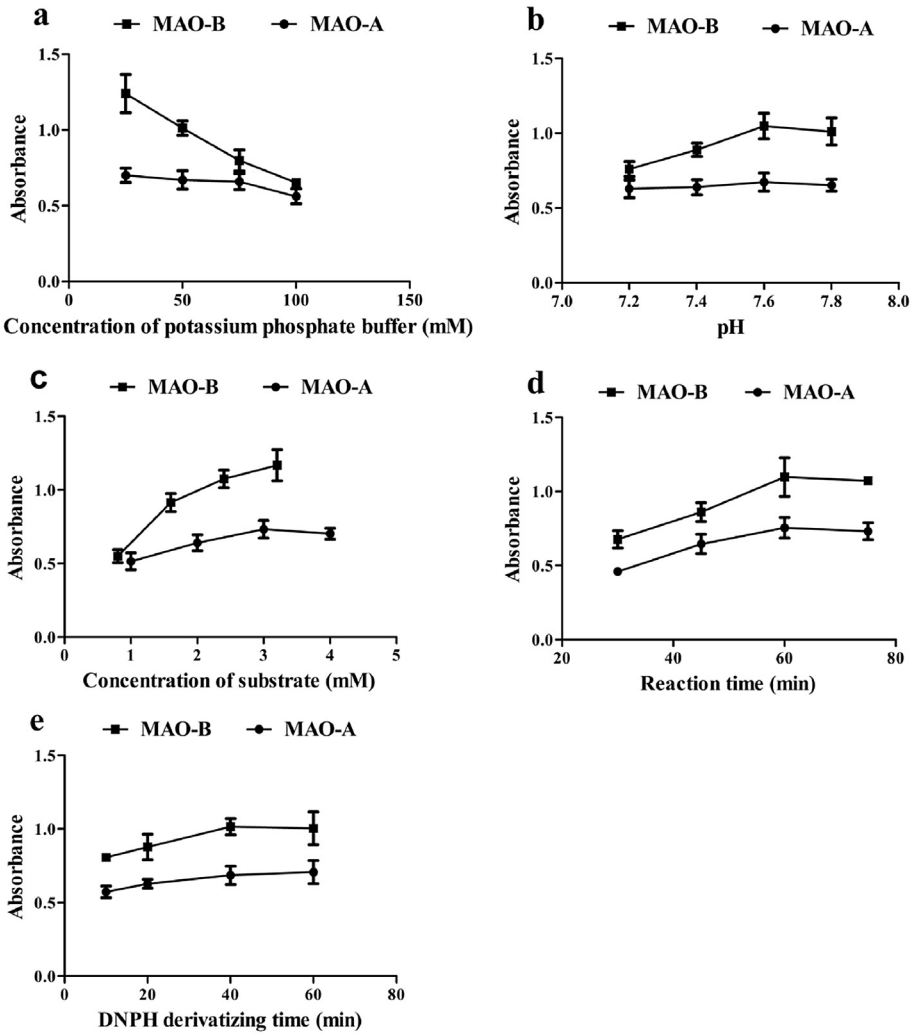

Fig. 3. Effects of reaction conditions of DNPH spectrophotometry on MAO activity. a, concentration of potassium phosphate buffer; b, pH of potassium phosphate buffer; c, concentration of substrate (benzylamine or 5-HT); d, reaction time and e DNPH derivatizing time. The absorbance are the mean of four samples and the results are shown as mean ± SD (n = 4).

absorbance by DNPH spectrophometric assay is approximately 2–3 times more sensitive by direct spectrophotometric assay, because benzaldehyde and 5-hydroxyindole acetaldehyde are derivatized by DNPH to form the corresponding DNPHydrazones and quinone anion that have large conjugated structures with the transitions $\pi \rightarrow \pi^*$ (Fig. 1). This results in stronger UV absorptions compared to the underivatized products. This behavior is similar to reported calibration curves. Fig. 5 shows that lower MAO concentrations are required for DNPH spectrophotometry to achieve the same absorbance in the two assays. In order to achieve an absorbance of 0.2–0.8, the MAO protein concentration required was approximately 47.5 and 77.5 μg for MAO-B and MAO-A, respectively, by DNPH spectrophotometry; while MAO protein concentration was 132 and 270 μg for MAO-B and MAO-A, respectively, by direct spectrophotometry. Although the sensitivity of DNPH spectrophotometry is lower than ELISA [20,21], fluorophotometry [22,23] and radioisotope assay [7], the radioisotope assay is time-consuming and expensive, while flurophotometry is more likely to be affected by the fluorescent interference of test compounds. Based on luminescent signals, the bioluminescent assay has been developed for assaying MAO activity; and is well-suited for high-throughput screening inhibitors [11]. Furthermore, the bioluminescent assay could increase sensitivity and reduce interference with external fluorescence; but expensive luciferin detection reagents (esterase and luciferase) are needed. Moreover, MAO-B and MAO-A activities were constructed in the presence of specific inhibitors or specific MAO-A/-B proteins. In addition, HPLC could improve sensitivity and specificity, but it is more expensive and laborious [24–26]; and the HPLC column has a limited life time if guanidine hydrochloride is used to dissolve the DNPhydrazone compound [14,27]. More than 205 and 90 min are needed in the traditional DNPH spectrophotometry reported by Basha [18] and direct spectrophotometry, respectively; and our method only required 105 min. Therefore, this proposed method has advantages in time and experimental operation, and is more sensitive, when compared to direct spectrophotometry.

4.4. Analysis of the inhibition of rasagiline for MAO in vitro

This proposed DNPH spectrophotometric method was used to determine the inhibition of rasagiline, in order to reflect MAO activity in rat livers and brains in vitro. The change on maximum absorbance was associated with the interaction between rasagiline and enzymes (Fig. 7). IC_{50} values were calculated by SPSS, and are

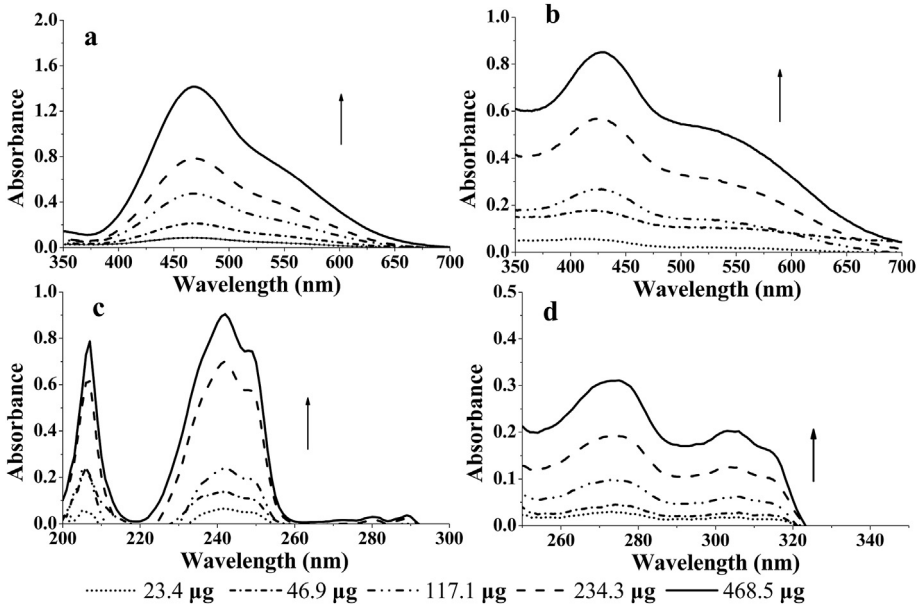

Fig. 4. The increasing concentration of MAO protein results in a corresponding increasing in absorbance by DNPH spectrophotometric method (a,b) and direct spectrophotometric method (c,d).

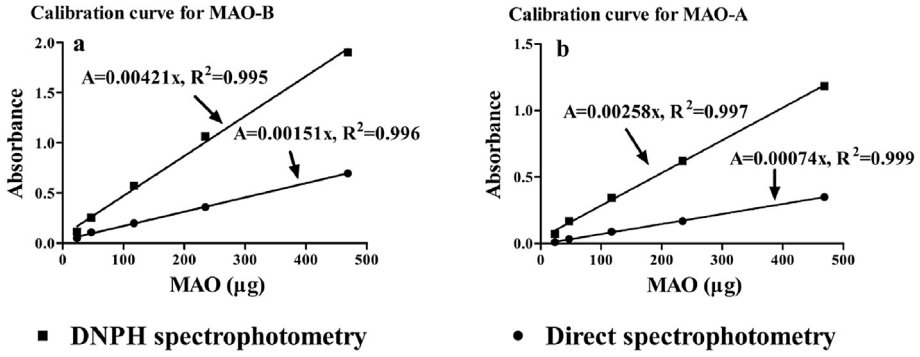

Fig. 5. Comparison of calibration curves for DNPH spectrophotometry (square) and direct spectrophotometry (circle). From left to right, the loads were 23.4, 46.9, 117, 234, 469 μg of MAO protein. The graph shows that the regression equation for MAO-B and MAO-A are A = 0.00421x, R^2 = 0.995 and A = 0.00258x, R^2 = 0.997 by DNPH spectrophometry and A = 0.00151x, R^2 = 0.996 and A = 0.00074x, R^2 = 0.999 by direct spectrophotometry.

Table 2
Repetition and precision analysis of DNPH spectrophotometry.

Methods	Tissues	Repetition		Precision	
		Mean ± SD	RSD	Mean ± SD	RSD
DNPH spectrophotometry	Liver MAO-B	1.53 ± 0.06	3.92%	1.45 ± 0.01	0.69%
	Liver MAO-A	0.840 ± 0.06	7.14%	0.897 ± 0.03	3.34%
	Brain MAO-B	1.21 ± 0.09	7.44%	1.24 ± 0.01	0.81%
	Brain MAO-A	1.07 ± 0.05	4.67%	1.02 ± 0.01	0.98%
Direct spectrophotometry	Liver MAO-B	0.792 ± 0.01	1.26%	0.788 ± 0.02	2.54%
	Liver MAO-A	0.307 ± 0.01	3.26%	0.304 ± 0.01	3.29%
	Brain MAO-B	0.681 ± 0.05	7.34%	0.723 ± 0.02	2.77%
	Brain MAO-A	0.371 ± 0.03	8.09%	0.392 ± 0.01	2.55%

The equal volume tissue homogenates were incubated for 20 min at 37 °C before addition of benzylamine (for MAO-B) or 5-HT (for MAO-A) and continuously incubated 60min, MAO activities were determined by DNPH spectrophotometry and direct spectrophotometry, and the absorbances are given as mean ± SD (n = 5). The RSD is presented as the following equation: RSD (%) = (SD × 100)/mean.

presented in Table 3. In order to compare the reliability of this proposed assay, the inhibition of rasagiline for MAO activity was also measured by direct spectrophotometry. There were no significant differences in IC_{50} ($P > 0.05$) between the DNPH and direct spectrophotometry methods. Rasagiline was approximately 60 times more potent for the inhibition of MAO-B compared with MAO-A in both DNPH and direct spectrophotometry. In addition, IC_{50} values of rasagiline by DNPH spectrophotometry were 8.00×10^{-9} M for MAO-B and 2.59×10^{-7} M for MAO-A; while IC_{50} values of rasagiline were 4.43×10^{-9} M for MAO-B and

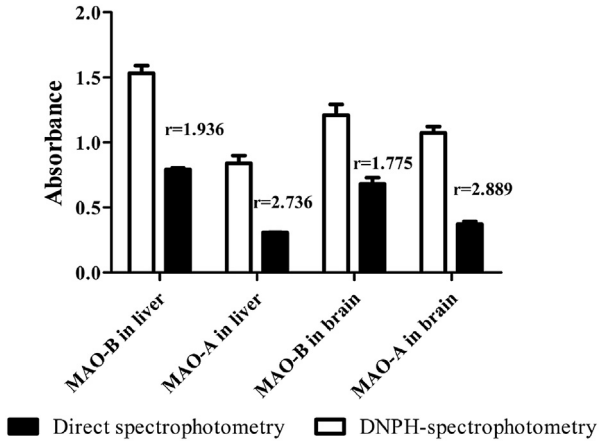

Fig. 6. An equal volume of liver or brain homogenate was incubated with substrate 37 °C and the MAO-B and MAO-A activities were determined with DNPH spectrophotometry and direct spectrophotometry. The absorbance is presented as mean ± SD (n = 5) and the ratio (r) shows the increased sensitivity by DNPH spectrophotometry compared to direct spectrophotometry.

4.12×10^{-7} M for MAO-A by a radioisotope method, as reported in published literature [28,29]. These are shown in Table 4. These indicate that the IC_{50} values and selectivity were in good agreement with the range of the reported and obtained values using the radioisotope assay. It is worth noting that DNPH spectrophotometry has the potential to be widely used, since it does not need a radioisotope and expensive instruments [12,13]. Thus, our method has the potential to measure MAO activity even in large samples, and could be widely applied for screening MAOIs.

Table 4
Published IC_{50} (M) values by different methods.

Tissue	MAO-B	MAO-A	Reference
Rat brain	$4.43 \pm 0.92 \times 10^{-9}$	$4.12 \pm 1.23 \times 10^{-7}$	MBH. Youdim [28]
Rat brain	$4 \pm 0.001 \times 10^{-9}$	$0.41 \pm 0.12 \times 10^{-6}$	H Zheng [7]
Human brain	$1.4 \pm 0.35 \times 10^{-8}$	$7.1 \pm 0.93 \times 10^{-7}$	MB.H. Youdim [28]
Human brain	1.4×10^{-8}	7.10×10^{-7}	JJ. Chen [29]
Rat brain	2.5×10^{-9}	7.3×10^{-8}	JJ. Chen
Rat brain	2.3×10^{-9}	1.49×10^{-7}	JJ. Chen
Rat brain	4.43×10^{-9}	4.12×10^{-7}	JJ. Chen
F344/N rats	$2.1 \pm 0.6 \times 10^{-6}$		MK Hu [23]

MAO activity inhibitions by rasagiline were published and taken from reference.

5. Conclusion

In this study, we developed a sensitive and simple spectrophotometry to detect MAO activity using DNPH as a derivatizing agent. The DNPH spectrophotometry is suitable for measuring MAO with low activity or at a low concentration. This method could be potentiality used to evaluate IC_{50} values of inhibitors with the following advantages: (1) compared to direct spectrophotometry, this method is more sensitive for measuring MAO with low activity or concentration, since DNPhydrazones have a larger conjugated structure and stronger UV absorption; (2) by adding NaOH, the alkalization of this reaction system shifts the absorption wavelength from 370 nm to 425/465 nm, which eliminates interference from free DNPH; (3) this method shortens the experimental time, since the application of an alkaline solution simplifies the tedious procedures of precipitating, washing and redissolving processes; (4) this method avoids the use of organic solvents and expensive or sophisticated equipment, showing that this method has certain advantages in experimental

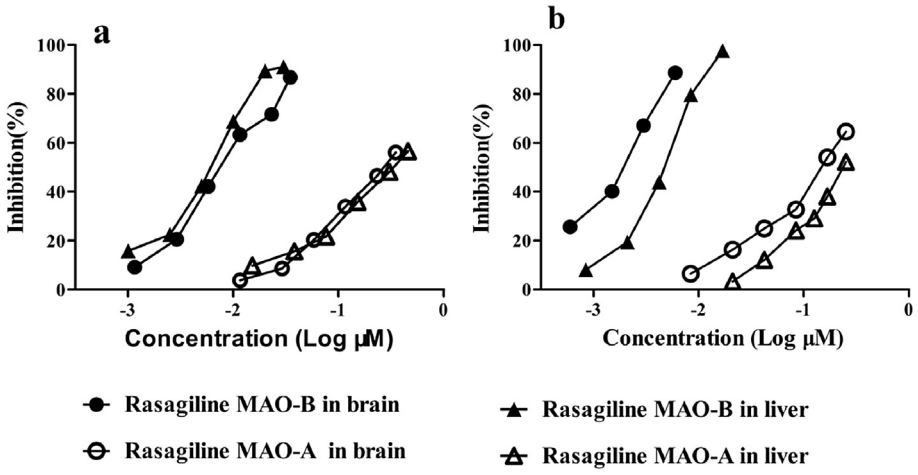

Fig. 7. In vitro inhibition of rasagiline against Sprague-Dawley (SD) rat liver and brain MAO. Rasagiline was added at concentration of 10^{-7} M to 10^{-9} M. Then the solution was incubated with liver and brain homogenates for 20 min at 37 °C before adding benzylamine or 5-HT and incubated continuously 60 min. Then absorbance was determined by DNPH spectrophotometry (a) and direct spectrophotometry (b). The inhibition was calculated as the equation: inhibition(%) = $(1 - A/B) \times 100\%$.

Table 3
IC_{50} values (M) for inhibition of MAO-B and MAO-A by rasagiline in SD rat liver and brain in vitro.

Methods	Liver		Brain	
	MAO-B	MAO-A	MAO-B	MAO-A
DNPH spectrophotometry	$5.00 \times 10^{-9*}$	$3.38 \times 10^{-7*}$	$8.00 \times 10^{-9*}$	$2.59 \times 10^{-7*}$
Direct spectrophotometry	5.80×10^{-9}	3.97×10^{-7}	8.00×10^{-9}	2.11×10^{-7}

The inhibition by rasagiline at various concentrations was measured by DNPH spectrophotometry and direct spectrophotometry. IC_{50} values were calculated using 5–7 concentrations of rasagiline by SPSS program, $^*P > 0.05$: no significant difference between DNPH spectrophotometry and direct spectrophotometry.

studies when compared with other methods.

In conclusion, this proposed method possesses properties of simplicity, sensitivity, safety and rapidity. It is suitable for measuring MAO activity, and has the potential to be used for screening MAOIs in many laboratories.

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