Key Laboratory of Nuclear Medicine, Ministry of Health, Jiangsu Institute of Nuclear Medicine, Wuxi, Jiangsu, P.R. China

Extractive spectrophotometric determination of TRODAT-1 hydrochloride in lyophilized kit

X. M. Li, Z. P. Chen, S. P. Wang, J. Tang, C. Y. Liu, M. F. Zou

Received April 2, 2008, accepted April 14, 2008

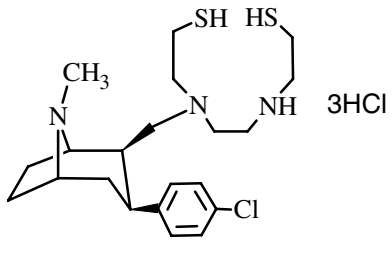
Zhengping Chen, Ph.D., Key Laboratory of Nuclear Medicine, Ministry of Health, Jiangsu Institute of Nuclear Medicine, No. 20 Qianrong Road, Wuxi 214063, P.R. China
chenzhengping@hotmail.com

Pharmazie 63: 638–640 (2008) *doi: 10.1691/ph.2008.8095*

A simple, sensitive, and accurate spectrophotometric method has been developed for the assay of TRODAT-1 hydrochloride in lyophilized kit. The method is based on the formation of ion-pair association complex of TRODAT-1 with bromothymol blue (BTB) in disodium hydrogen phosphate/citric acid buffer of pH 4.0. The colored product was extracted with chloroform, and measured spectrophotometrically at 414 nm. Beer's law was obeyed in the range of 5–25 μg/ml with molar absorptivity of 2.75×10^4 l/mol/cm. Optimization of experimental conditions was described for the method. The proposed method has been successfully applied for the analysis of TRODAT-1 hydrochloride in lyophilized kit. No interference with pharmaceutical excipients was observed.

1. Introduction

TRODAT-1, ([2-[2-[[[3-(4-chlorophenyl)-8-methyl-8-azabicyclo[3,2,1]oct-2-yl]methyl](2-mercaptoethyl)amino]ethyl]-amino]ethanethiolate[3-]-oxo-[1R-(exo-exo)]), is a cocaine analogue that can be labeled with technetium-99m to produce ^{99m}Tc-TRODAT-1. The ^{99m}Tc-labeled tropane derivative, ^{99m}Tc-TRODAT-1, is a radiopharmaceutical used in brain receptor imaging, which binds to the dopamine transporter (DAT) selectively and specifically (Dresel et al. 1998; Fang et al. 2000b; Hwang JJ et al. 2002; Kung et al. 1997; Meegalla et al. 1997). In the last 10 years, ^{99m}Tc-TRODAT-1 has been shown to be an useful DAT imaging agent in the diagnosis of Parkinson's disease (Chou et al. 2004; Huang et al. 2004; Huang et al. 2001; Hwang et al. 2004; Kao et al. 2001; Mozley et al. 2000; Tzen et al. 2001; Wang et al. 2005). It is prepared by adding ^{99m}Tc sodium pertechnetate to a sterile lyophilized kit that contains TRODA-1 hydrochloride and other excipients. Like other pharmaceuticals, the quality control of the active ingredient has an important impact on the clinical effectiveness and safety of the use of radiopharmaceuticals. So, the need to establish a quantitative assay for measuring TRODAT-1 hydrochloride content in lyophilized kit is becoming increasingly urgent. Up to now, to our knowledge, no method has been reported for its determination in kit.



TRODAT-1

In this study, a simple, sensitive, and accurate spectrophotometric method was presented for the determination of TRODAT-1 hydrochloride in lyophilized kit, which can be used for routine quality control analysis. This method is based on the formation of an ion-association complex between TRODAT-1 and bromothymol blue (BTB) in disodium hydrogen phosphate/citric acid buffer of pH 4.0. The proposed method could be incorporated into the routine pharmaceutical quality control program that may be applied to the process and to the final product.

2. Investigations, results and discussion

Among the methods available for the quantitative determination of pharmaceutical compounds, extractive spectrophotometric procedure continues to be a popular method for its simplicity, sensitivity, and economical advantages (Cardoso and Milano 2005; Cardoso et al. 2007; El-Didamony 2008; Erk 2004; Rahman et al. 2004). In the present work, an extractive spectrophotometric method was developed for the determination of TRODAT-1 hydrochloride in lyophilized kit. TRODAT-1 reacted with BTB in acidic buffer to yield a chloroform soluble ion-association complex, which exhibited absorption maximum at 414 nm. Under the experimental conditions, the corresponding reagent blank exhibited a negligible absorbance. The absorption spectra is shown in the Fig.

A number of preliminary experiments were performed to determine the optimum reaction conditions for the assay procedure, and the optimum variables were maintained throughout the experiments. First, the effect of pH was studied by extracting the colored complexes in the presence of disodium hydrogen phosphate/citric acid buffer. The maximum colour intensity and constant absorbances were observed in the buffer with pH in the range of 3.9–4.2. Further, 5.0 ml buffer gave maximum absorbances

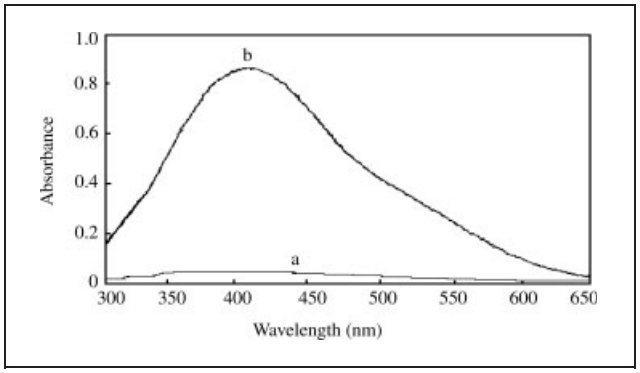


Fig.: Absorption spectrum of reagent blank (a) and ion-association complex of TRODAT-1 (12.5 μg/ml) with BTB (b)

Table 1: Optical characteristics, precision, and accuracy data

Parameter	BTB
λ_{max} (nm)	414
Beer's law limits (μg/ml)[a)]	5–25
Molar absorptivity (l mol^{-1} cm^{-1})	2.75×10^4
Sandell's sensitivity (μg cm^{-2})	1.98×10^{-2}
Stability (h)	2
Regression equation (Y)[b)]	
Slope, b ± S.D.	0.0045 ± 0.0008
Intercept, c ± S.D.	0.0219 ± 0.0091
Correlation coefficient (r) ± S.D	0.9980 ± 0.0036
Limit of detection (μg/ml)	1.02
Limit of quantification (μg/ml)	3.40

a) Average of six determinations
b) Y = bX + c, where X is the concentration of drug in μg/ml

Table 2: Determination of TRODAT-1 hydrochloride in lyophilized kit by the proposed method

Sample	Amount of TRODAT-1 (μg)		Recovery (n = 3, %)
	Added	Found[a)]	
1	50.0	50.34 ± 1.95	100.69
2	50.0	48.79 ± 2.24	97.58
3	50.0	49.59 ± 1.72	99.18

a) Average of 3 determinations ± standard deviation

and reproducible results. For these reasons, 5.0 ml buffer of pH 4.0 was chosen for all subsequent experiments. Second, the effects of different reagents were investigated by measuring the absorbances of solutions containing a fixed concentration of TRODAT-1 and varied amounts of the respective reagent. It was found that the maximum absorbance was attained with 3.5 ml BTB, and above this volume the absorbance remained unchanged. Therefore, 4.0 ml BTB was selected for further investigation. Third, the effect of different extraction solvents on the ion-pair complexes was also studied. A quantitative recovery of the complexes was achieved with 8.0 ml chloroform, and one extraction was adequate. Therefore, 8.0 ml chloroform was used for further studies. Finally, it was observed that shaking times of 1.5 to 3 min produced constant absorbances. Therefore, the optimum shaking time was fixed at 2 min throughout the experiment.

The stability of the sample solutions was monitored by keeping the solutions at room temperature and then the absorbances of the solutions were measured every 15 min. These measurements revealed the absorbances of the sample solutions was unchanged for at least 2 h at room temperature.

The accuracy of the proposed method was presented by performing recovery experiments through standard addition method. In view of this, known amounts of pure TRODAT-1 were added to pre-analyzed formulations at three different concentration levels and the mixtures were analyzed. The results demonstrated that the average percent recoveries and relative standard deviation (RSD) values were in the range of 98.4–101.5% and 0.81–1.23%, demonstrating good accuracy of the method. It was found that there was no interference from any excipients that presented in lyophilized kit, such as $SnCl_2 \cdot 2\,H_2O$, $EDTA \cdot 2\,Na$, $Na_2HPO_4 \cdot 12\,H_2O$, KH_2PO_4, and sodium glucoheptonate (GH).

The precision of analytical procedure was investigated by intra-day and inter-day determinations of three different concentrations of TRODAT-1 hydrochloride in five replicates. The intra and inter-day RSD values were found to be 0.88–2.06%, and indicated reasonable repeatability of the proposed method.

Under the optimized experimental conditions, the Beer's law limit, molar absorptivity, Sandell's sensitivity value, regression equation, and correlation coefficient are displayed in Table 1. Beer's law is obeyed for TRODAT-1 in the concentration range of 5–25 μg/ml with molar absorptivity 2.75×10^4 l/mol/cm. The Sandell's sensitivity of the color system is found to be 1.98×10^{-2} μg/cm^2. The linearity of the calibration graph is excellent with a correlation coefficient of 0.9980. The limit of detection (LOD = 3SD/b) and limit of quantification (LOQ = 10 SD/b) of this method are 0.58 and 1.93 μg/ml, respectively (IUPAC 1978), where SD is the standard deviation of reagent blank (n = 5) and b is the slope of the calibration curve.

In order to examine the applicability of the proposed method in routine pharmaceutical analysis, we have applied this method to determining the TRODAT-1 hydrochloride content in lyophilized kit. Three replicate determinations have been made. As shown in Table 2, the amount of TRODAT-1 in the kit is 49.57 ± 1.97 μg/kit (labeled amount is 50.0 μg/kit). The closeness of the results to the labeled amount indicates the accuracy of our method. According to the analysis results of the lyophilized kit, there is no interference from the excipients. All these suggests that this proposed method is satisfactory for the analysis of TRODAT-1 hydrochloride in lyophilized kit.

The lyophilized kit including TRODAT-1 hydrochloride and other excipients was dissolved by 5.0 ml saline solution to obtain a solution of pH 7.38. Therefore, 5.0 ml disodium hydrogen phosphate/citric acid buffer of pH 3.58 was added to the lyophilized kit, and then a solution was obtained at a final pH of 4.0.

In conclusion, in this present work, we reported an extractive spectrophotometric method to quantitate TRODAT-1 hydrochloride in lyophilized kit. The significant advantage of this method is that it can be applied to determine a small dose drug for its high sensitivity. The proposed method offers good linearity and precision, and it can be applied to the analysis of a wide concentration range of TRODAT-1 hydrochloride in the kit with satisfactory results. Furthermore, the method is simple and inexpensive, and it does not require any pretreatment of the kit. Therefore, the proposed method could be incorporated into the routine pharmaceutical quality control program that may be applied to the process and to the final product.

3. Experimental

3.1. Chemicals and reagents

All of the chemicals used were of analytical reagent grade; distilled water was used throughout. TRODAT-1 and the lyophilized kit containing 50.0 μg TRODAT-1 were synthesized by ourselves (Chen et al. 2006; Fang et al. 2000a). Aqueous solutions of BTB (0.05%), TB (0.05%) and BCG (0.05%) were prepared in distilled water. Disodium hydrogen phosphate/citric acid buffer of pH 3.0–6.0 were prepared by the standard method. Standard solution of TRODAT-1 (1.0 mg/ml) was prepared in ethanol and stored in a refrigerator.

3.2. Apparatus

A BECKMAN DU-600, UV/Vis spectrophotometer (USA) with 1.0 cm quartz cell was used for the absorbance measurements. The pH value was determined by a BECKMAN pH 72 digital pH meter (USA).

3.3. Methods

Aliquots of 40.0 to 200.0 μL of standard TRODAT-1 solution (1.0 mg/ml) corresponding to 40.0 to 200.0 μg were transferred into a series of 30 ml separating funnels. Chloroform (8.0 ml), BTB (4.0 ml), and disodium hydrogen phosphate/citric acid buffer of pH 4.0 (5.0 ml) were added to each funnel. The contents of each funnel were shaken well for 2 min and left at room temperature for 25 min. The chloroform layer was separated and then passed through anhydrous sodium sulphate. The absorbances of the yellow colored complexes were measured at 414 nm, against the reagent blank.

Chloroform (8.0 ml) and BTB (4.0 ml) were taken into a series of 30 ml separating funnels. Every two lyophilized kits were dissolved by 5.0 ml disodium hydrogen phosphate/citric acid buffer of pH 3.58, and the solution of pH 4.0 was converted into each 30 ml separating funnel. The assay was following the procedure given above. The content of TRODAT-1 hydrochloride in lyophilized kit was calculated from the corresponding linear regression equation.

Acknowledgements: This work was supported by the National Natural Science Foundation of P.R.China (30570518); High Technology Research and Development Program of Jiangsu Province of China (BG2007603); Science Foundation of Health Department of Jiangsu Province of China (H200401)

References

Cardoso SG, Milano J (2005) Spectrophotometric determination of oxiconazole in topical lotion using methyl orange. J Pharm Biomed Anal 37: 639–642.

Cardoso SG, Ieggli CV, Pomblum SC (2007) Spectrophotometric determination of carvedilol in pharmaceutical formulations through charge-transfer and ion-pair complexation reactions. Pharmazie 62: 34–37.

Chen ZP, Wang SP, Li XM, Zhang TX, Tang J (2006) Development of a new kit formulation of $^{99}Tc^{m}$-TRODAT-1. Chin J Nucl Med 26: 235–237.

Chou KL, Hurtig HI, Stern MB, Colcher A, Ravina B, Newberg A, Mozley PD, Siderowf A (2004) Diagnostic accuracy of [^{99m}Tc]TRODAT-1 SPECT imaging in early Parkinson's disease. Parkinsonism Relat Disord 10: 375–379.

Dresel SH, Kung MP, Plössl K, Meegalla SK, Kung HF (1998) Pharmacological effects of dopaminergic drugs on in vivo binding of [^{99m}Tc]TRODAT-1 to the central dopamine transporters in rats. Eur J Nucl Med 25: 31–39.

El-Didamony AM (2008) Spectrophotometric determination of benzydamine HCl, levamisole HCl and mebeverine HCl through ion-pair complex formation with methyl orange. Spectrochim Acta Part A 69: 770–775.

Erk N (2004) Spectrophotometric determination of indinavir in bulk and pharmaceutical formulations using bromocresol purple and bromothymol blue. Pharmazie 59: 183–186.

Fang P, Chen ZP, Zhou X (2000a) Synthesis of ^{99m}Tc-TRODAT-1 as dopamine transporter imaging agent. Chin J Pharm 31: 244–247.

Fang P, Wu CY, Liu ZG, Wan WX, Wang TS, Chen SD, Chen ZP, Zhou X (2000b) The preclinical pharmacologic study of dopamine transporter imaging agent [^{99m}Tc]TRODAT-1. Nucl Med Biol 27: 69–75.

Huang CC, Yen TC, Shih TS, Chang HY, Chu NS (2004) Dopamine transporter binding study in differentiating carbon disulfide induced Parkinsonism from idiopathic Parkinsonism. NeuroToxicology 25: 341–347.

Huang WS, Lin SZ, Lin JC, Wey SP, Ting G, Liu RS (2001) Evaluation of early-stage Parkinson's disease with ^{99m}Tc-TRODAT-1 imaging. J Nucl Med 42: 1303–1308.

Hwang JJ, Liao MH, Yen TC, Wey SP, Lin KJ, Pan WH, Chen JC, Ting G (2002) Biodistribution study of [^{99m}Tc] TRODAT-1 alone or combined with other dopaminergic drugs in mice with macroautoradiography. Appl Radiat Isot 57: 35–42.

Hwang WJ, Yao WJ, Wey SP, Ting G (2004) Reproducibility of ^{99m}Tc-TRODAT-1 SPECT measurement of dopamine transporters in Parkinson's disease. J Nucl Med 45: 207–213.

IUPAC (1978) Nomenclature, symbols, units and their usage in spectrochemical analysis – II. Spectrochim Acta Part B 33: 242.

Kao PF, Tzen KY, Yen TC, Lu CS, Weng YH, Wey SP, Ting G (2001) The optimal imaging time for [$^{99}Tc^{m}$]TRODAT-1/SPET in normal subjects and patients with Parkinson's disease. Nucl Med Commun 22: 151–154.

Kung MP, Stevenson DA, Plössl K, Meegalla SK, Beckwith A, Essman WD, Mu M, Lucki I, Kung HF (1997) [^{99m}Tc]TRODAT-1: a novel technetium-99m complex as a dopamine transporter imaging agent. Eur J Nucl Med 24: 372–380.

Meegalla SK, Plössl K, Kung MP, Chumpradit S, Stevenson DA, Kushner SA, McElgin WT, Mozley PD, Kung HF (1997) Synthesis and characterization of technetium-99m-labeled tropanes as dopamine transporter-imaging agents. J Med Chem 40: 9–17.

Mozley PD, Schneider JS, Acton PD, Plössl K, Stern MB, Siderowf A, Leopold NA, Li PY, Alavi A, Kung HF (2000) Binding of [^{99m}Tc]TRODAT-1 to dopamine transporters in patients with Parkinson's disease and in healthy volunteers. J Nucl Med 41: 584–589.

Rahman N, Khan NA, Azmi SNH (2004) Extractive spectrophotometric methods for the determination of nifedipine in pharmaceutical formulations using bromocresol green, bromophenol blue, bromothymol blue and eriochrome black T. Il Farmaco 59: 47–54.

Tzen KY, Lu CS, Yen TC, Wey SP, Ting G (2001) Differential diagnosis of Parkinson's disease and vascular Parkinsonism by ^{99m}Tc-TRODAT-1. J Nucl Med 42: 408–413.

Wang J, Jiang YP, Liu XD, Chen ZP, Yang LQ, Liu CJ, Xiang JD, Su HL (2005) ^{99m}Tc-TRODAT-1 SPECT study in early Parkinson's disease and essential tremor. Acta Neurol Scand 112: 380–385.