Rat Plazma Örneklerinde Yüksek Basınçlı Sıvı Kromatografisi (HPLC) ile Yeni Bir Monopiridinyum Oksim Olan TS-131'in Ölçümü

Measurement of TS-131, a New Monopyridinium Oxime, by High Performance Liquid Chromatography in Rat Plasma Samples

Kromatografi / Chromatography

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Özet

Amaç

TS-131, mevcut ticari oksimlere potansiyel bir alternatif olarak geliştirilmiş bir monopiridinyum aldoksim tipi kolinesteraz reaktivatörüdür. Rat plazma örneklerinde TS-131'in konsantrasyonlarının ölçümü için; sensitif, basit ve güvenilir bir yüksek basınçlı sıvı kromatografisi (HPLC) yöntemi kurmayı amaçladık.

Gereç ve Yöntemler

TS-131, erkek Sprague Dawley ratlara intramusküler olarak verildi. 30 dakika sonra kan örnekleri intrakardiyak olarak alındı. TS-131'in HPLC ile ayırımı için, octadecyl silica yapısında analitik kolon ve %92 0.1 M amonyum asetat ve %8 metanol içeren mobil faz kullanıldı. Tüm analizler 40 ^{0}C'ye ayarlanmış kolon fırını içerisinde yapıldı. Analizler, diyot array dedektör ile 242 nm dalga boyunda yapıldı.

Bulgular

3200 µmol/L'lık stok standarttan seri dilüsyonlarla elde edilen standartlarla (0.78–3200 µmol/L) hesaplanan kalibrasyon eğrisi lineerdi. Bu HPLC yöntemi ile dedekte edilebilen TS-131 aralığı 0.78–3200 µmol/L arasında idi. Yöntemin kantitasyon limiti (LOQ)'u 0.39 µmol/L'ydi. HPLC ölçümlerinin gün-içi ve günler-arası tekrarlanabilirlilikleri sırasıyla %1.94 ve %1.22 idi. Standartın numunelere eklenmesinden (spiking) sonra, ortalama spike geri eldeleri %99.2 ile %100.4 arasındaydı. Ortalama geri elde ise %99.8 olarak bulundu.

Sonuç

TS-131 ölçümü için; sensitif, basit ve güvenilir bir HPLC yöntemi kuruldu. Mevcut diyod array dedektörlü HPLC yöntemi, aynı zamanda çeşitli piridinyum oksimlerin konsantrasyonlarını belirlemek için de kullanılabilir.

Anahtar Kelimeler

TS-131, Oksim, Kolinesteraz Reaktivatörü, HPLC, Rat.

Abstract

Aim

TS-131 is a monopyridinium aldoxime-type cholinesterase reactivator developed as a potential alternative to commercially available oximes. A sensitive, simple and reliable high performance liquid chromatography (HPLC) method with diode array detector was developed for the measurement of TS-131 concentrations in rat plasma samples.

Material and Methods

Male Spraque Dawley rats were treated intramuscularly with TS-131 and the samples were collected 30 min. later. Separation was carried out by HPLC using octadecyl silica stationary phase and a mobile phase consisting of 92% 0.1 M ammonium acetate and 8% methanol. Measurements were carried out at 40 ^{0}C. Quantitative absorbance was monitored at 242 nm.

Results

The calibration curve was linear through the range of 0.78–3200 µmol/L, which is well beyond the detected plasma level range of TS-131. Limit of quantitation was 0.39 µmol/L. Intra-day and inter-day precisions of the HPLC determinations gave standard deviations as 1.94 and 1.22%, respectively. After spiking, average spike recoveries ranged from 99.2% to 100.4% and, overall mean recovery of 99.8% was found.

Conclusion

A sensitive, simple and reliable HPLC method was established for the measurement of TS-131. The presented HPLC method with diyote arrray detector can also be used to determine the concentrations of several pyridinium oximes.

Keywords

TS-131, Oxime, Cholinesterase Reactivator, HPLC, Rat.

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Introduction

Organophosphates and organophosphonates (organophosphorus compounds, OPs) are serine esterase and protease inhibitors. OPs are widely used in all over the world in agriculture as pesticides, insecticides and acaricides, in industry and technology as softening agents and additives to lubricants [1-3]. They cause an irreversible inhibition of cholinesterases (butyrylcholine esterase: EC 3.1.1.8 and acethylcholine esterase: EC 3.1.1.7) via a covalent reaction with the serine in the active center of the enzymes [1,2}. Some OPs as tabun, sarin, cyclosarin, soman, VR and VX are highly toxic "nerve gas" warfare agents. Some of the warfare agents have been also misused by terrorist groups. The clinical effects of OPs are well known and have been extensively studied and described [3-5].

The reactivators of acethylcholine esterase (AChE) are an essential part of antidote treatment during OPs intoxication, together with atropine and diazepam [6]. The reactivator carries a nucleophilic oxime group (an oximate anion in the dissociated form) which is able to cleave the OPs moiety from AChE's active site and restore the enzyme's vital function [7]. Pralidoxime (1,2-hydroxyiminomethyl-1-methylpyridinium chloride) and obidoxime (3, Toxogonin, 1,3-bis(4-hydroxyiminomethylpyridinium)-2-oxapropane dichloride) are commercially available AChE reactivators. On the other hand, Atropine is a necessary antidote component to diminish the cholinergic consequences of OPs intoxication, where diazepam is used for its anticonvulsive activity [6,7].

However, the commercially available reactivators cannot be used to treat the full spectrum of OPs. In particular, few of the compounds are effective against tabun and pesticide intoxication [8]. Novel reactivators are being developed worldwide to cover these deficiencies [9-15].

The available oximes have been quantitatively determined using high performance liquid chromatography (HPLC). Using this technique, it was possible to monitor the blood concentration and urinary elimination of pralidoxime [16-22]. Urinary elimination of obidoxime was followed by reversed-phase chromatography. Detection of obidoxime and of the structurally similar other oximes was carried out at 258 nm [20] and 304 nm [22].

A new AChE reactivator was developed by the authors as a potential alternative to the commercially available substances. Its chemical structure was derived from the structures of existing esterase reactivators, especially pralidoxime. The newly developed oxime is monopyridinium aldoxime. It is the so-called TS-131. Chemically, it is 3-carboxyaldehydepyridinium oxime, C6H6N2O, with a molecular weight of 122.134 g/mol. Its chemical structure is given in Figure 1, and its 1H NMR spectra is shown in Figure 2. TS-131 has a maximum absorbance at 242 nm.

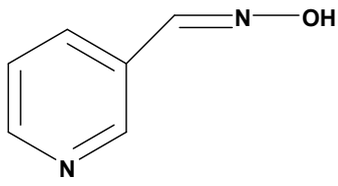


Figure 1. The chemical structure of TS-131 (3-carboxyaldehydepyridinium)

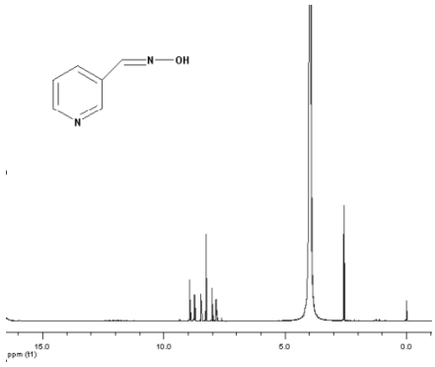
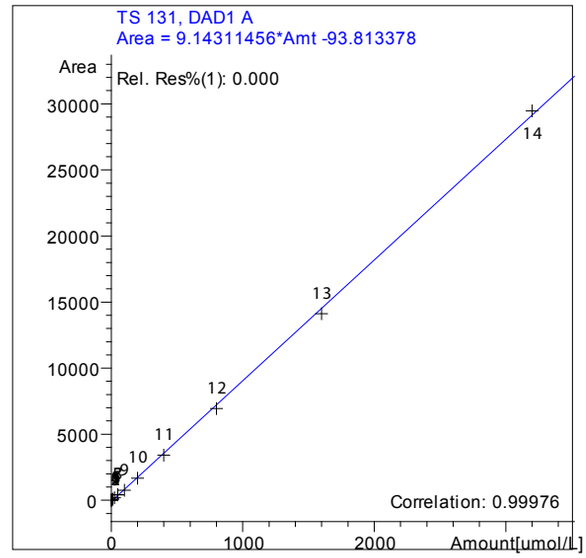


Figure 2. The 1H NMR spectra of TS-131.



Şekil 3. Calibration curve for TS-131 measurement when TS-131 solution was prepared with HPLC grade water. Each point represents average of three measurements. The linearity was evaluated by regression analysis performed by the least-squares method.

The main aims of this study were the determination of TS-131 in rat plasma samples and to establish a simple and reliable method.

Material and Methods

Animals

In the study, 20 adult male Spraque Dawley rats (250 to 300 gr) were used. Rats were caged in a controlled environment at 22°C with 12-hour light/dark cycles. Standard rat feed and reverse-osmosis–purified water were provided ad libitum. All rats were allowed to have 1 week of acclimation to this environment before the experiment. The rats were divided into groups of ten animals. The present study was performed in the Research Center of the Gulhane Military Medical Academy with the approval of the Gulhane Military Medical Academy Animal Ethics Committee, Turkey.

Chemicals

TS-131 (3-carboxyaldehydepyridinium, with the molecular formula of C6H6N2O, and the molecular weight of 122.134) was synthesized in the laboratory (Department of Chemistry, Middle East Technical University, Ankara, Turkey). Other chemicals were purchased from commercial sources in the best available quality: methanol, HPLC grade (Merck, Darmstadt, Germany); ammonium acetate, trichloroacetic acid, (Sigma-Aldrich Chemie GmbH, Steinheim, Germany). The water was double distilled and deionized and of HPLC grade (Millipore, Molsheim, France).

HPLC System

The liquid chromatography system consisted of an Agilent 1100 Autosampler, a HP 1050 HPLC Quaternary Pump, and a HP 1050 Diode

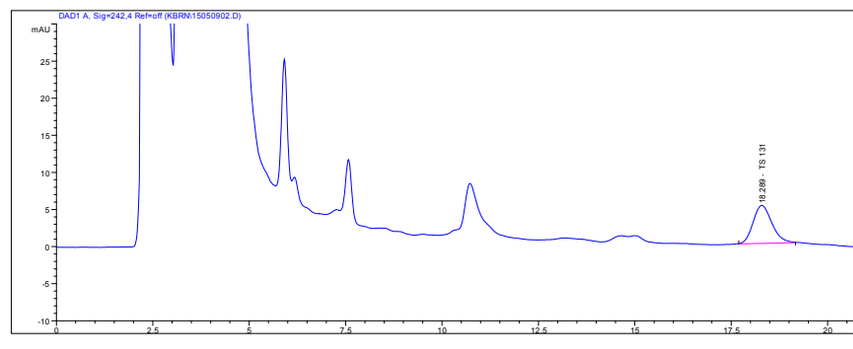


Figure 4. Plasma concentration of TS-131 following intramuscularly (i.m.) administration as a single dose (300 µmol/kg), 13.20 µmol/L TS-131 at 18.29 min. The peaks not marked are unknown oxidable endogenous compounds.

Array Detector (DAD), HP 1050 solvent/Degassing Module, HP Thermostatted column Compartment all from Agilent Technologies (Waldbronn, Germany). The 3D ChemStations Software of 1100 System Access served for evaluation of chromatograms. A Waters Spherisorb ODS2 separation column (25 cm × 4.6 mm i.d., particle size: 5 µm) of Hichrom (Berkshire, England), containing octadecyl silica as stationary phase was used. The mobile phase consisted of 8% methanol and 92% 0.1 M ammonium acetate. The mobile phase flow rate was 1 mL/min with isocratic mode. The chromatograms were obtained at 40 ^{0}C. The size of sample of injection was 50 µL. Ultraviolet absorbance was detected at 242 nm.

Animal treatment

300 µmol/kg of TS-131 was intramuscularly (i.m.) injected to male Spraque Dawley rats. The rats were sacrificed after 30 min. following i.m. administration. In addition, a control group which did not receive any treatment was also used. The blood was collected in K3EDTA coated tubes (Becton, Dickinson, NJ, USA), gently mixed and centrifuged at 3500 rpm for 10 min at 4 ^{0}C to obtain the plasma. The plasma samples were stored at −80 ^{0}C until HPLC analysis.

Plasma pretreatment

Plasma sample aliquots (0.5 mL) were mixed with 0.5 mL of 20% trichloroacetic acid to precipitate the proteins, and centrifuged at 13.000 g for 10 min at 4 ^{0}C. The supernatant was then injected into the HPLC system.

Calibration

Calibration curve was established. Calibration curve was determined using 14 dilutions from a 3200 µmol/L TS-131 stock solution prepared with HPLC grade water in the range of 0.78–3200 µmol/L, in triplicate. The linearity was evaluated by regression analysis performed by the least-squares method and gave R2 = 0.9997. During the day, the TS-131 solution was stabile (100±2% SD). Endogenous compounds in the biological samples separated well from TS-131 that migrated with a retention time of 18.29 min.

Results

We described a high performance liquid chromatographic method with diode array detector for measurement of TS-131 in rat plasma samples. As shown in Figure 3, the calibration curve for TS-131 dissolved in HPLC grade water was linear in the range of 0.78–3200 µmol/L, and the limit of quantitation was 0.39 µmol/L. When plasma concentrations of TS-131 in rats were studied following intramuscular administration of this compound, a HPLC with DAD method was used with isocratic mode (flow rate: 1 mL/min) and UV detection at 242 nm.

The calibration curve was determined using 14 calibrators obtained from a 3200 µmol/L TS-131 stock solution prepared with HPLC grade water. Regression analysis performed by the least-squares method yielded the equation y = 9.14311456x−93.813378 and a correlation coefficient of R2 = 0.9997. The required sample pretreatment is simple, and the measurement is rapid with a retention time of only 18.29 min.

A simple protein precipitation serves the clean-up before the HPLC injection. This protein precipitation method is quite well for the determination of TS-131 in the plasma samples. Intra-day and inter-day precisions were expressed in standard deviation of the TS-131 HPLC determination, and gave 1.94 and 1.22%, respectively. After spiking of TS-131 into control rat plasma, average spike recovery ranged from 99.2% to 100.4% and, overall mean recovery of 99.8% was found. The NMR spectrum of TS-131 has verified its chemical structure.

Plasma level of TS-131 was determined in rats. In the plasma samples of rats treated i.m. with 300 µmol/kg TS-131, and the 30 min post-treatment concentration was 13.20 µmol/L value (Figure 4).

Discussion

In especially animal studies, esterase reactivators as pralidoxime and obidoxime are commonly used to protect from mortality against several OPs like paraoxon and tabun. In this area, different groups are working on newly developed pyridinium oximes. Lorke et al. [23] have shown that to protect from death, two new asymmetric bispyridinium oximes (K-27 and K-48) are much more efficient than pralidoxime [24] and obidoxime [25].

Several techniques including high-performance liquid chromatography have been reported to be able to measure concentrations of different cholinesterase reactivator oximes in plasma. Kalasz et al. have developed a HPLC-EC assay for measuring K-27 in different biological samples [26]. On the other hand, a HPLC-UV method was established to determine concentrations of K-27 and K-48 in plasma and brain tissue samples by Lorke et al. [23]. Guyon et al. [18] determined pralidoxime concentrations by HPLC from samples of rat plasma and urine.

A sensitive, simple and reliable HPLC method was established for the measurement of TS-131. The presented HPLC method with DAD can also be used to determine the concentrations of several pyridinium oximes.

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