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Determination of the impurity profile of 1,2-cyclohexanedione dioxime by high-performance liquid chromatography

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Abstract

High-performance liquid chromatography (HPLC) was used to determine the impurity profile of 1,2-cyclohexanedione dioxime (CDO), a key ingredient in the radiopharmaceutical CardioTec® kit (kit for the preparation of ^{99m}Tc teboroxime). The HPLC assay separated CDO from potential impurities which included 1,2-cyclohexanedione, cyclohexanone monoxime and cyclohexanedione monoxime (CDM). The method employed a mobile phase consisting of 0.1% phosphoric acid–acetonitrile (82:18, v/v), a Hamilton 10-μm PRP-X100 anion-exchange column (250 × 4.1 mm I.D.), and UV absorbance detection (238 nm), and achieved a resolution (R_s) ≥ 1.5 for the three detected impurities: 1,2-cyclohexanedione, cyclohexanone monoxime and cyclohexanedione monoxime (CDM) in the presence of CDO. The method was improved compared to existing methods by achieving a rapid, simultaneous separation (15 min) of compounds not previously reported and quantitating impurities at the 0.2–5% (w/w) level of sensitivity.

1. Introduction

1,2-Cyclohexanedione dioxime (CDO) is a key component in CardioTec®, a kit for the preparation of the radiopharmaceutical myocardial imaging agent ^{99m}Tc teboroxime. ^{99m}Tc teboroxime is formed in situ in the kit by the combination of CDO, methyl boronic acid, a chlorine atom and ^{99m}Tc atom, upon the addition of ^{99m}Tc sodium pertechnetate and heating for 15 min at 100°C [1,2]. It was necessary to establish the impurity profile of CDO to monitor both the overall quality and the equivalence of material from different manufacturers.

Commercially, CDO raw material is usually manufactured by the oxidation of cyclohexanone with selenium dioxide or selenous acid to form 1,2-cyclohexanedione, followed by oximation using hydroxylammonium chloride in a basic solution [3,4]. Potential impurities from the reaction include: the starting material, cyclohexanone; the intermediate product, 1,2-cyclohexanedione; and the side reaction products cyclohexanone monoxime and cyclohexanedione monoxime. The reaction scheme and the chemical structures for CDO and these potential impurities are shown in Fig. 1.

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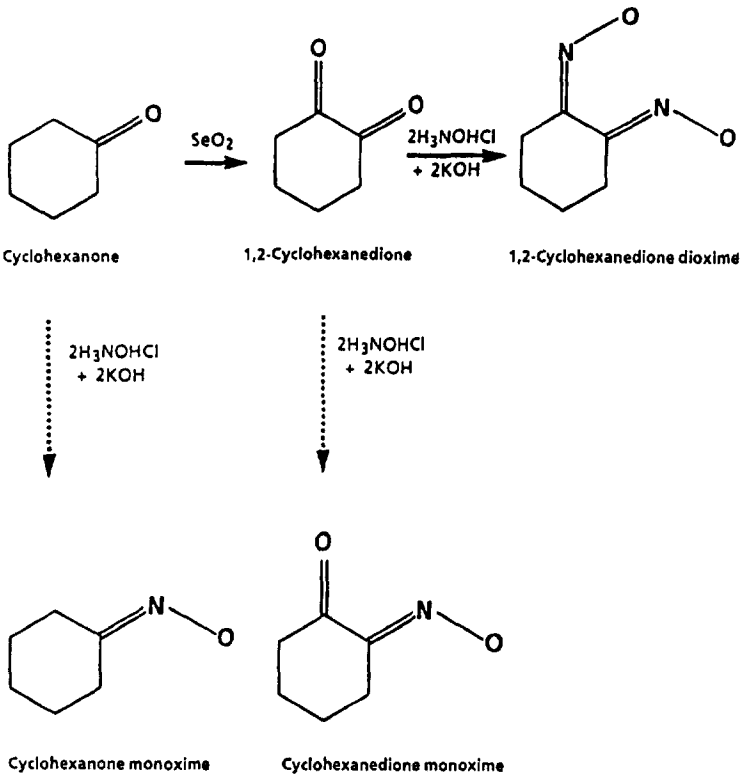

Fig. 1. Structures for CDO and potential synthetic pathway impurities.

Several polarographic [5–8], spectrophotometric [9–12], atomic absorption [13–15] and liquid chromatographic [16–18] methods for the determination of oximes can be found in the literature. However, most are indirect or unsuitable for routine analysis. Various high-performance liquid chromatographic (HPLC) methods have been applied for carbonyl compound analysis using micro [19], specialized C_{18} [20–25], polymeric [23] and anion-exchange columns [23] with UV–Vis [19–22,24,26] and fluorescence [23,25] detection. Gas chromatographic methods have been applied for carbonyl compound analysis using several means of detection such as flame ionization [27,28], thermal conductivity [29] and thermionic specific detection [30]. Deeler and Hendricks [18] achieved baseline resolution of two, but not all, of the components addressed in this study, and needed a chemical reaction system coupled to the chromatographic column to achieve separation and detection. Jandera and Churacek [26] stated that aldehydes and ketones are not ionized in aqueous solutions and separation by anion exchangers can be achieved either by complex formation of the analytes with the positive charge of the exchanger or by the differences in solubilities in aqueous–organic solutions. Some studies also indicated that ketones, which are able to form enols with slightly acidic character, can be sorbed on strongly basic anion-exchange resins as the oxime group has a slightly basic nitrogen atom and a mildly acidic hydroxyl group and can react as a conjugate base [26,31,32]. Thus, anion-exchange high-performance liquid chromatography is particularly effective for the separation of the oximes under investigation, and PRP-X100, a poly(styrene–divinylbenzene) trimethylammonium anion exchanger, which provides physical and chemical stability over the pH range 1–13 with a solvent compatibility range of 0–100% for organic and aqueous buffers, was chosen as optimal for the analysis.

An HPLC method was developed to rapidly

and simultaneously separate CDO raw material from its potential impurities, generated as intermediates or side-reaction products. The method was specifically optimized for the detection of CDM, the singularly most common impurity in CDO. This paper describes the chromatographic separation and includes limits of detection, response factors of the impurities and quantitation of CDO.

2. Experimental

2.1. Materials

CDO was obtained from Fluka Chemika-BioChemika (Buchs, Switzerland) and GFS Chemicals (Powell, OH, USA), cyclohexanone monoxime and 1,2-cyclohexanedione were obtained from Fluka Chemika-BioChemika and cyclohexanedione monoxime was supplied by Bristol-Myers Squibb Radiopharmaceutical Research Department. Acetonitrile and methanol were HPLC grade from Burdick and Jackson/Baxter Scientific Products (McGaw Park, IL, USA). The water was HPLC/organic-free from the NANOpure II system from SYBRON/Barnstead (Boston, MA, USA).

The HPLC/data collection system from Spectra-Physics (Piscataway, NJ, USA) consisted of a Model 4270 integrator, a Model 8800 pump, and a Model 8780 autosampler with a 20-μl (nominal) fixed loop injector all on a LABNET data collection system, and a Kratos Spectroflow 757 variable-wavelength UV–Vis detector set at 238 nm from Applied Biosystems (Foster, CA, USA). A Hamilton 10-μm PRP-X100 anion-exchange column, 250 × 4.1 mm I.D. (Baxter Scientific Products) was used with a mobile phase of 0.1% phosphoric acid–acetonitrile (82:18, v/v) pumped at a flow-rate of 1.0 ml/min at ambient temperature.

2.2. Methods

System suitability

Prior to use, the column was conditioned by pumping mobile phase at 1.0 ml/min for 1 h. A system-compatible solution containing 8 μg/ml each of CDO and 1,2-cyclohexanedione, 80 μg/ml of cyclohexanone monoxime, and 2 μg/ml of cyclohexanedione monoxime (CDM) was prepared in methanol and injected in order to determine the component response factors. The resolution, $R_s = 2(t_2 - t_1)/(W_1 + W_2)$, between the peaks was ≥ 1.5.

Intra-assay precision

A CDO standard solution (8 μg/ml) was prepared in methanol and injected 10 times.

Reproducibility

Two CDO standard solutions (8 μg/ml) were prepared in methanol and injected 15 times each over a 5-day period.

Potency

CDO sample solutions (8 μg/ml) were prepared in methanol and injected in duplicate.

Linearity

Five CDO standard solutions (1–9.6 μg/ml) were prepared in methanol and injected in duplicate. Linear regression analysis was performed on the standard curve generated by plotting the CDO concentration versus peak-area response (data points were not averaged prior to regression analysis).

Standard recovery

A solution containing CDM (0.45 μg/ml), cyclohexanedione (0.77 μg/ml) and cyclohexanone monoxime (8.1 μg/ml) in methanol was spiked with CDO to yield solutions with CDO concentrations of 6.4, 8.0, and 9.6 μg/ml.

3. Results and discussion

Initially, CDO and its four potential impurities were injected onto the chromatographic system to determine the retention time of each. How-

Table 1
Molar extinction coefficients at λ_{max} for CDO and its impurities

Compound	λ_{max} (*nm*)	log ϵ
1,2-Cyclohexanedione dioxime [33]	232	4.12
Cyclohexanedione monoxime	238	3.76
1,2-Cyclohexanedione [34]	265	3.42
Cyclohexanone monoxime [35]	189	3.89
Cyclohexanone [36]	282	1.23

ever, at 238 nm, cyclohexanone does not absorb strongly and could not be detected even when injected neat. A comparison of the molar extinction coefficients at λ_{max} for CDO and its four potential impurities is shown in Table 1. A chromatogram of an injection of CDO and the three detectable impurities produced baseline resolution of all components, $R_s \geq 1.5$ (Fig. 2). The resolution between CDO and 1,2-cyclohexanedione, the closest eluting component, was 2.6. Relative response factors, estimated limits of detection ($S/N = 2$), and retention times are given in Table 2. The response factors for the impurities increased with increasing retention time. Limits of detection were most sensitive for the fast eluting, sharp peaks (i.e., CDM and 1,2-cyclohexanedione) and were least sensitive for the long eluting, broad peak (i.e., cyclohexanone monoxime).

To show the applicability of the method, multiple sample lots of CDO raw material from

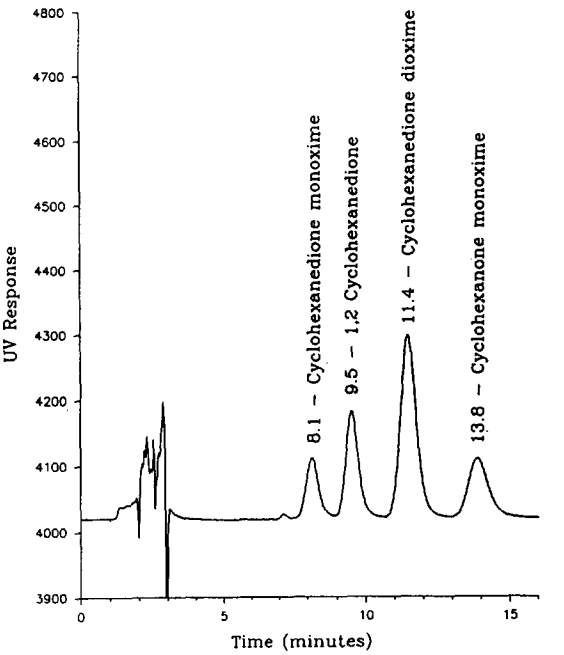

Fig. 2. Chromatogram of CDO and three potential synthetic pathway impurities using a PRP-X100 column, 0.1% phosphoric acid–acetonitrile (82:18, v/v) at 1.0 ml/min, and 238 nm UV detection.

two manufacturers (I and II) were assayed for CDO potency (Table 3). In all cases, the potency is less than 100%; however, CDM and moisture account for total mass balance within experimental error. The practical application of the method is graphically displayed in Fig. 3, showing overlaid chromatograms of a CDO raw

Table 2
HPLC UV detection parameters for CDO and its impurities (λ = 238 nm)

Compound	Relative response factor[a]	Detectable weight on column (μg)[b]	Limit of detection (%w/w)[c]	Retention time (min)
Cyclohexanedione monoxime	1.18	0.004	0.2	8.1
1,2-Cyclohexanedione	2.16	0.007	0.4	9.5
1,2-Cyclohexanedione dioxime	1.0	0.003	0.2	11.4
Cyclohexanone monoxime	26.9	0.08	5.0	13.8

[a] Relative to CDO as (compound concentration/compound area)/(CDO concentration/CDO area).
[b] Estimated based on peak responses of weight loads of 0.009, 0.0154, 0.0192 and 0.08 μg for CDM, 1,2-cyclohexanedione, CDO, and cyclohexanone monoxime, respectively, when signal-to-noise ratio equals 2 ($S/N = 2$).
[c] Estimated relative to maximum injectable amount of 1.6 μg CDO on the column.

Table 3
Potency and impurity index data of CDO from two manufacturers

Sample number	Manufacturer	Potency (% anhy.)	Moisture (%)	Impurity index (%)	Impurities found
1	I	98.8	0.1	0.9	CDM
2	I	97.7	0.4	0.9	CDM
3	I	98.2	0.1	0.6	CDM
4	II	99.1	0.3	0.7	CDM
5	II	97.9	0.4	0.8	CDM
6	II	98.9	0.2	0.8	CDM

material containing the CDM impurity (0.9% impurity index) and a CDO standard.

To validate the method, intra-assay precision, reproducibility, CDO standard linearity, and CDO standard recovery were examined. Ten replicate injections of a single CDO working standard (8 μg/ml) yielded peak area responses of 61 370–63 051 with a mean of 62 180, standard deviation of 549, and coefficient of variation of 0.9%, indicating excellent intra-assay precision. Fifteen injections each of two CDO working standards (each standard 8 μg/ml), yielded response factors of 6754–7525 with a mean of 7142, standard deviation of 274, and coefficient of variation of 3.8%, indicating good injection-to-injection reproducibility. The relationship between peak response and the concentration of CDO standard was linear over the range 1–9.6 μg/ml, representing 12.5–120% of the working standard concentration. Linear regression analysis yielded a correlation coefficient of 0.9999 and P-value < 0.001. The equation of the line was $y = 6778x - 1$, with standard deviations of 40 and 239 for the slope and intercept, respectively, and 95.0% confidence limits for the slope at 6686 and 6871. CDO standard recoveries of 98.9–100.7% were obtained when a solution containing CDM (0.45 μg/ml), cyclohexanedione (0.77 μg/ml) and cyclohexanone monoxime (8.1 μg/ml) impurities was spiked with CDO to yield solutions with CDO concentrations of 6.4, 8.0, and 9.6 μg/ml. These standards represented 80, 100, and 120% of the 8 μg/ml CDO working standard, respectively (Table 4).

Ruggedness testing indicated that approximately 250 injections could be made on the column over a period of 20 days with no detectable loss of resolution. The use of a water–acetonitrile (1:1) rinse cycle of one hour for column clean-up is recommended in order to eliminate possible column contamination, thereby extending the column lifetime.

In conclusion, this HPLC method provided a profile of three of four potential impurities from the CDO synthetic pathway. The method is rapid (15 min) and sensitive for simultaneously

Table 4
Recovery of CDO in the presence of CDM, 1,2-cyclohexanedione and cyclohexanone monoxime

CDO added (μg/ml)	Recovery (%)		
	Cyclohexanedione monoxime	1,2-Cyclohexanedione	Cyclohexanone monoxime
6.4	100.5	100.6	100.7
8.0	99.6	99.9	100.5
9.2	98.9	99.4	100.3
Mean recovery	99.7	100.0	100.5
Coefficient of variation (%)	0.8	0.6	0.2

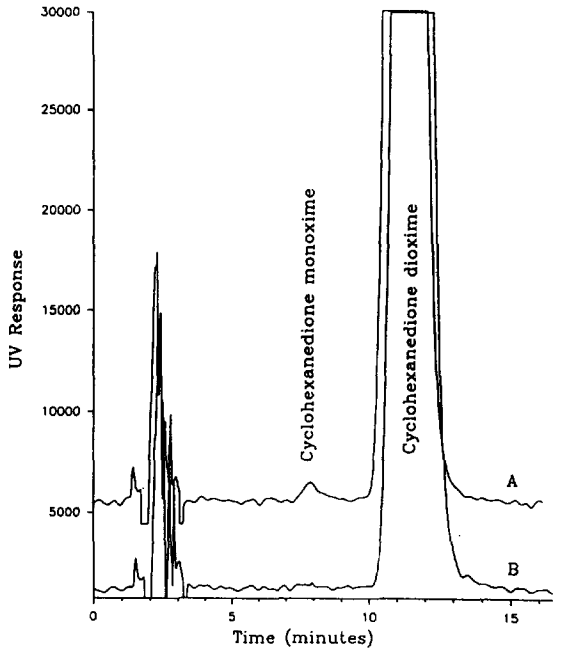

Fig. 3. Chromatograms of a CDO raw material containing the CDM impurity (0.9% impurity index) (A) and a CDO standard (B), using a PRP-X100 column, 0.1% phosphoric acid–acetonitrile (82:18, v/v) at 1.0 ml/min, and 238 nm UV detection.

quantitating impurities at the 0.2–5% (w/w) level, when injecting 1.6 μg of CDO, and is suitable for determining CDO potency due to its ability to eliminate potential impurity interferences.

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