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# Liquid chromatographic determination of oxaliplatin in blood using post-column derivatization in a microwave field followed by photometric detection

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**Abstract**

Oxaliplatin ([(1*R*,2*R*)-1,2-cyclohexanediamine-*N*,*N*′]oxalato(2-)-*O*,*O*′-platinum) is the first platinum drug with significant activity for metastatic colon cancer. The analysis of oxaliplatin has previously almost exclusively been based on the determination of the platinum content in plasma or ultrafiltrate using flameless atomic absorption spectroscopy (FAAS) or inductively coupled plasma mass spectrometry (ICPMS). A new method for quantitative determination of the free fraction of the intact drug in blood ultrafiltrate is presented here. Blood was ultrafiltrated centripetally at 4 °C and the ultrafiltrate was analyzed by liquid chromatography. Oxaliplatin was separated on a Hypercarb column using a mobile phase of methanol/succinic acid buffer pH 7.0 (9/1, v/v). Post-column derivatization was performed by adding *N*,*N*-diethyldithiocarbamate in methanol and with microwave heating of a Teflon tubing. The derivative was quantified by photometric detection at 344 nm. The coefficient of variation of standard blood samples was 4.9 and 2.5% at 0.100 and 1.00 μg/ml, respectively. The limit of quantitation was 0.04 μg/ml.


*Keywords:* Derivatization, LC; Oxaliplatin

## 1. Introduction

Oxaliplatin ([(1*R*,2*R*)-1,2-cyclohexanediamine-*N*,*N*′]oxalato(2-)-*O*,*O*′-platinum) is a novel platinum complex which in combination with other chemotherapeutics has a significant cytostatic activity for metastatic colon cancer [1]. The pharmacokinetics of oxaliplatin has so far almost exclusively been based on analysis of the platinum content in plasma and ultrafiltrate using flameless atomic absorption spectroscopy (FAAS) or inductively coupled plasma mass spectrometry (ICPMS) [2]. However, analytical techniques measuring the concentration of platinum will codetermine the parent compound as well as other cytotoxic and biologically inactive biotransformation products. Recently, the kinetics of oxaliplatin in plasma ultrafiltrate from patients was studied using liquid chromatography followed by fraction collection and measuring the platinum content by FAAS [3].

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We here describe a rapid liquid chromatographic method for the determination of the free fraction of oxaliplatin in whole blood using on-line post-column derivatization in a microwave field.

## 2. Experimental

### 2.1. Chemicals

All chemicals were of analytical grade and were commercially available products. Oxaliplatin was a generous gift from Sanofi (Malvern, PA, USA).

### 2.2. Equipment

The LC analysis was carried out utilizing a Valco Model C6W injector (Houston, TX, USA) with a fixed loop volume of 25 μl, a LC-10AD Shimadzu pump (Kyoto, Japan), and a Shimadzu SPD-10AVvp UV-Vis detector (Kyoto, Japan) measuring at 344 nm. The post-column reagent was provided by a LC-10AD Shimadzu pump. The column and reagent flows were mixed in a T-piece obtained from Upchurch Scientific (Oak Harbor, WA, USA) with a low dead volume (1.5 μl) connected to a Teflon tubing (length: 2.3 m, i.d.: 0.51 mm, o.d.: 1.57 mm). Two meters of the tubing were placed in the microwave field lined around a Teflon holder obtained from AT-Maskin AB (Uppsala, Sweden). The microwave heating was performed using a Smithcreator from Personal Chemistry (Uppsala, Sweden). The output signal from the detector was processed in a Chromeleon 6.20 chromatography data system from Dionex (Sunnyvale, CA, USA).

The liquid chromatographic fraction containing the derivative was collected and analyzed separately by electrospray mass spectrometry (MS) in the positive mode using a Fisons VG Platform instrument (Altrincham, UK).

### 2.3. Chromatographic and post-column derivatization conditions

The liquid chromatographic separations were performed at room temperature with a Hypercarb (250 mm × 4.6 mm i.d., particle size 5 μm) column obtained from ThermoHypersil (Runcorn, UK) using a mobile phase of methanol/succinic acid buffer 0.25 M pH 7.0 (9/1, v/v) with a flow rate of 0.5 ml/min. The post-column derivatization was performed by mixing the mobile phase with 2.7 mM sodium *N*,*N*-diethyldithiocarbamate in methanol (flow rate: 0.17 ml/min). The microwave heating was set to a temperature of 110 °C corresponding to an effect of approximately 130 W. The temperature was monitored by the IR device in the instrument with the measuring area being located about (1/4) from the Teflon tubing outlet.

### 2.4. Sample preparation

Blood (2 ml) was collected in prechilled Vacutainer® tubes, stored on ice and dispensed in prechilled Centrisart®I 10,000 MW cut-off filter from Sartorius AG (Göttingen, Germany) and ultrafiltrated centripetally (20 min, 4 °C, 4000 × *g*) within 1 h. The ultrafiltrates were stored at −80 °C and analyzed within 3 weeks. Standard curves were prepared by adding oxaliplatin to blood with a hematocrit appropriate to the samples to be analyzed. The blood standards (0.04–2.5 μg/ml, $n = 6$) were run in parallel with each patient samples. The degradation of oxaliplatin was <10% during the preparation and storing conditions.

## 3. Results and discussion

Liquid chromatography with porous graphitic carbon (PGC) has previously been utilized for the preparative isolation [4–6] and quantitiative analysis [7–9] of Pt-containing cytotoxic agents in pure aqueous solutions. In the present paper, PGC is successfully used for the separation of oxaliplatin in blood ultrafiltrate.

Post-column derivatization with sodium diethyldithiocarbamate (DDTC) has been used for the determination of cisplatin and its monohydrated complex in biological fluids using a packed-bed reactor [10]. Preliminary studies have also indicated that monomodal microwave heating could be used instead to affect the derivatization yield [11]. In the present study, the post-column reaction of oxaliplatin with DDTC was carried out in a microwave field. Initially MicroWell 10 from Labwell (Uppsala, Sweden) was used for

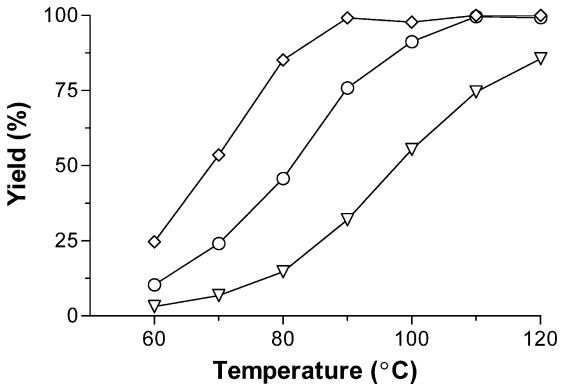


Fig. 1. Yield of the $Pt(DDTC)_2$ derivative. (◇) $1.5 \times 10^{-3}$ M DDTC; (○) $7.0 \times 10^{-4}$ M DDTC; (▽) $3.0 \times 10^{4}$ M DDTC.

the microwave heating but since it is no longer commercially available the present results were obtained using the Smithcreator. The results obtained were the same for both microwave devices. Fig. 1 gives the yield of the $Pt(DDTC)_2$ derivative as a function of the temperature estimated by IR monitoring at the outside of the Teflon tubing, and the concentration of DDTC in the reactor. The structure of the derivative was established by electrospray MS run in the positive mode showing ions at $[M]^+$ and $[M + Na]^+$ with the expected isotope distribution. The yields were calculated using synthetically prepared $Pt(DDTC)_2$ as a reference compound [10]. As expected, the reaction is faster when the concentration of the reagent is increased. However, the detector noise is more profound and therefore analysis of the blood samples was carried out using a temperature of 110 °C and a reagent concentration in the reactor of $7.0 \times 10^{-4}$ M. Fig. 2 gives a chromatogram from analysis of patient blood containing 0.64 μg/ml of oxaliplatin and blood from the same patient immediately before treatment. No peaks were observed in the blank which would interfere with the quantification of oxaliplatin. The biotransformation products: the dichlorocomplex of oxaliplatin, the chloro oxalato monodentate complex [12], the oxalato monodentate intermediate [8] and the dihydrated complex [7] had shorter retention times than oxaliplatin. The coefficient of variation of standard blood samples was 4.9 and 2.5% at 0.100 ($n = 5$) and 1.00 μg/ml ($n = 5$), respectively. The limit of

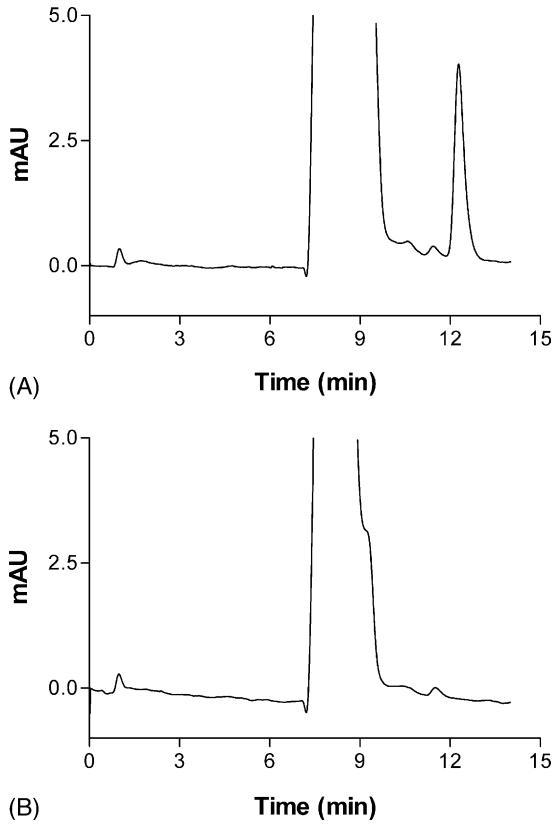


Fig. 2. Chromatogram from oxaliplatin analysis of blood from a patient under treatment (A) and blood from the same patient before treatment (B). Retention time for oxaliplatin: 12 min; oxaliplatin concentration: 0.64 μg/ml (A); DDTC concentration: $7.0 \times 10^{-4}$ M; temperature: 110 °C.

quantitation was 0.04 μg/ml with a signal-to-noise of 10. The system was robust and neither a change in the retention time of oxaliplatin nor the yield of the derivative was observed after injection of >500 blood ultrafiltrates. Linear regression analysis of standard curves obtained from blood with different hematocrit is presented in Table 1. A higher hematocrit resulted in a higher slope which indicates that the partition to the red blood cells was <1. Since the hematocrit shows profound interindividual variations in cancer patients it is important to take this into consideration when preparing standard samples. Fig. 3 shows the concentration of oxaliplatin in blood ultrafiltrate from a patient receiving oxaliplatin as a hepatic arterial infusion.

Table 1
Linear regression analysis of standard curves with different hematocrit

| Variable | Hematocrit | | |
|---|---|---|---|
| | 46 | 40 | 30 |
| Slope | 2.129 | 1.893 | 1.749 |
| ± S.E.M. | 0.047 | 0.024 | 0.039 |
| Intercept | −0.030 | −0.023 | −0.022 |
| ± S.E.M. | 0.024 | 0.012 | 0.020 |
| $r^2$ | 0.9961 | 0.9987 | 0.9961 |

$n = 10$ for each hematocrit, concentration range: 0.04–1.00 μg/ml.

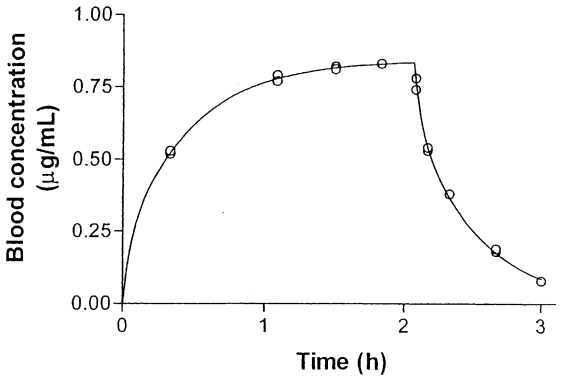


Fig. 3. Blood concentration time curve after hepatic arterial administration of oxaliplatin. Oxaliplatin dose: 104 mg/m$^2$; infusion time: 2 h.

In conclusion, a method is presented which provides for the first time a rapid robust technique for the determination of the free fraction of intact oxaliplatin in blood.

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## References

[1] J.L. Misset, H. Bleiberg, W. Sutherland, M. Bekradda, E. Cvitkovic, Crit. Rev. Oncol. Hematol. 35 (2000) 75.
[2] M.A. Graham, G.F. Lockwood, D. Greenslade, S. Brienza, M. Bayssas, E. Gamelin, Clin. Cancer Res. 6 (2000) 1205.
[3] S.S. Shord, S.A. Bernard, C. Lindley, A. Blodgett, V. Mehta, M.A. Churchel, M. Poole, S.L. Pescatore, F.R. Luo, S.G. Chaney, Anticancer Res. 22 (2002) 2301.
[4] H. Ehrsson, I. Wallin, A. Andersson, P.O. Edlund, Anal. Chem. 67 (1995) 3608.
[5] J. Yachnin, I. Wallin, R. Lewensohn, F. Sirzen, H. Ehrsson, Cancer Lett. 132 (1998) 175.
[6] A. Ekborn, A. Lindberg, G. Laurell, I. Wallin, S. Eksborg, H. Ehrsson, Cancer Chemother. Pharmacol. 51 (2003) 36.
[7] P. Videhult, J. Yachnin, E. Jerremalm, R. Lewensohn, H. Ehrsson, Cancer Lett. 180 (2002) 191.
[8] E. Jerremalm, P. Videhult, G. Alvelius, W.J. Griffiths, T. Bergman, S. Eksborg, H. Ehrsson, J. Pharm. Sci. 91 (2002) 2116.
[9] E. Jerremalm, S. Eksborg, H. Ehrsson, J. Pharm. Sci. 92 (2003) 436.
[10] A. Andersson, H. Ehrsson, J. Chromatogr. B 652 (1994) 203.
[11] H. Ehrsson, S. Stone-Elander, S. Moshashaee, A. Andersson, J.O. Thorell, N. Elander, J. High Resolut. Chromatogr. 17 (1994) 283.
[12] E. Jerremalm, M. Hedeland, I. Wallin, U. Bondesson, H. Ehrsson, in preparation.