Institute for Clinical Chemistry and Laboratory Medicine[1], Pharmacy[2], Jena University Hospital, Jena, Germany

Quality control of cytotoxic drug preparations by means of Raman Spectroscopy

M. KIEHNTOPF[1], B. MÖNCH[1], R. SALZER[1], M. KUPFER[2], M. HARTMANN[2]

Received June 26, 2011, accepted July 29, 2011

Dr. med. Dr. rer. nat. Michael Kiehntopf, Institute for Clinical Chemistry and Laboratory Medicine, Jena University Hospital, Erlanger Allee 101, 07740 Jena, Germany
michael.kiehntopf@med.uni-jena.de

Pharmazie 67: 95–96 (2012)
doi: 10.1691/ph.2012.1091

Quality control of drug preparations for prevention of medication errors is a prerequisite in the process of personalized drug dispensing. Current approaches are time consuming, laborious, personnel-intensive and not totally error free. Thus new, inexpensive, rapid and high qualitative error free quality control tools should be developed. The aim of this study was to use a direct, rapid and non-destructive Raman method for quantification of cytostatic drugs as a quality control measure in cytotoxic drug preparations. Raman spectra of aqueous solutions of 5-fluorouracil exhibit a dominant Raman band at approx. 766 cm^{-1}. The signal intensity, measured as 800–750 cm^{-1} peak area after baseline adjustment, correlates linear ($R^2 = 0.99487$) with 5-fluorouracil concentration. Precision was comparable with standard laboratory tests. Raman Spectroscopy can be applied for reliable quality control in the process of 5-fluorouracil drug preparations.

Prevention of medication errors in the process of personalized drug dispensing is important for patient safety. A recent study demonstrated that in a one year follow up 1.4% of 9952 dispensed doses at a university pharmacy department were mistaken (Pérez-Cebrián et al. 2011). Further European studies indicate that the rate of errors concerning intravenous administration are considerably higher than those involving medicines for oral use (Airaksinen et al. 2006). Therefore, it is obvious that monitoring of the final drug concentration becomes one of the most important parts of the quality control process in cytotoxic drug admixture services. According to clinical Good Manufacturing Practices (cGMP) dosing errors should be prevented by supervision of all stages of the drug preparation procedure. Often this is implemented by the four-eyes-principle, ensuring that e.g. dosage calculations and dosing were independently checked by another health care professional. However, this procedure is most notably personnel-intensive time consuming and error-prone. Therefore, alternative approaches for quality control are inevitable. The aim of our study was to evaluate the application of a direct, rapid and non-destructive Raman method for the quantification of cytostatic drugs as a quality control measure in cytotoxic drug preparations. It is well known that many drugs can be analyzed on the basis of their specific molecular inelastic light scattering characteristics by Raman Spectroscopy (Gordon and McGoverin 2011). Moreover, with the introduction of Surface Enhanced Raman Spectroscopy (SERS) it has been demonstrated that e.g. cytostatic drugs can be detected at very low concentrations in saliva (2 μg/ml 5-fluorouracil) as well as in blood plasma (1×10^{-8} M paclitaxel) (Farquharson et al. 2008; Yuen et al. 2010). Raman Spectroscopy has been already applied in the area of pharmaceutical analysis for process and quality control since this emerging analytical technique allows rapid, non-destructive quantification of active pharmaceutical ingredients and excipients in pharmaceutical tablets and capsules (Buckley and Matousek 2011).

In the past we used Raman Spectroscopy for identification of bacteria and cells (Harz et al. 2008, 2009) as well as for the detection of thiopurine methyl transferase activity (März et al. 2011). In this study we want to assess the applicability of Raman Spectroscopy as a quality control tool in the workflow of cytostatic drug preparations. In a first proof of principle experiment we analyzed an aqueous solutions of 5-fluorouracil 5-FU by Raman Spectroscopy. The obtained spectrum contains a dominant Raman band at approx. 766 cm^{-1}. This band is in accordance with previous results and can be assigned to the pyrimidine ring breathing mode (Farquharson et al. 2008). Next we analyzed aqueous solutions containing different concentrations of 5-fluorouracil, ranging from 0–5357 mg/l. We observed an increase of the 800–750 cm^{-1} peak area with increased concentrations of 5-fluorouracil (Fig. 1 A). The signal intensity, measured as 800–750 cm^{-1} peak area after baseline adjustment, is a function of the 5-fluorouracil concentration (Fig. 1 B). Figure 2 depicts a calibration curve, showing this linear correlation ($R^2 = 0.99487$). In order to evaluate the robustness of the method Raman measurements were repeated five times. Interassay CV from day to day was 2.6%, 2.0%, 1.4%, 3.0%, 4.7%, 2.8% and 10.5% at 5-fluorouracil concentrations of 5462.6 mg/l, 4477.0 mg/l, 2774.7 mg/l, 2376.7 mg/l, 1850.6 mg/l, 1456.4 mg/l and 801.1 mg/l, respectively. Thus, particularly at higher 5-FU concentrations precision was comparable with standard laboratory tests. Comparison of measured concentrations with calculated concentrations revealed a good agreement according to Passing-Bablok regression analysis (intercept -108.7 mg/l 95% CI -462.28 to 187.81; slope 1.02 95% CI 0.93 to 1.20).

The results presented in this proof of principle study demonstrate that Raman Spectroscopy can be applied for reliable quality control in the process of 5-fluorouracil drug preparations. 5-FU measurements can be accomplished at g/l concentrations in 2×90 s, demonstrating that this direct, rapid and non-destructive Raman method has the potential for routine application in hospital pharmacies and cytotoxic drug admixture services. Further evaluation of the method for quantification of other cytostatic drugs e.g. paclitaxel as well as validation in a routine setting under the same experimental conditions in independent pharmacies will be needed to clarify for which purposes Raman Spectroscopy should be used in the future for quality control and prevention of medication dispensing errors. Studies addressing these topics are under way and will hopefully bring us a step closer to a medication safety without any serious dispensing errors.

Experimental

1. Reagents and solutions

5-Flourouracil (2,4-dihydroxy-5-fluoropyrimidine) was obtained from Medac GmbH (lot number: C100163A) in a concentration of 50 mg/ml and used as prepared in the normal clinical setting.

5-FU concentrations from 0–8000 mg/l were diluted in 0.9% NaCl in the aseptic production rooms of the Pharmacy of the University Hospital Jena. This was in accordance with the product information of Medac. The following solutions were prepared: 500 mg, 700 mg, 1000 mg, 1200 mg, 1500 mg, 2500 mg and 3000 mg 5-FU were added to 500 ml 0,9% NaCl freeflex bags from Fresenius (lot number: 14DG7306). The final concentrations of 980 mg/l, 1362 mg/l, 1923 mg/l, 2290 mg/l. 2830 mg/l, 4545 mg/l and 5357 mg/l 5-FU were used for calibration.

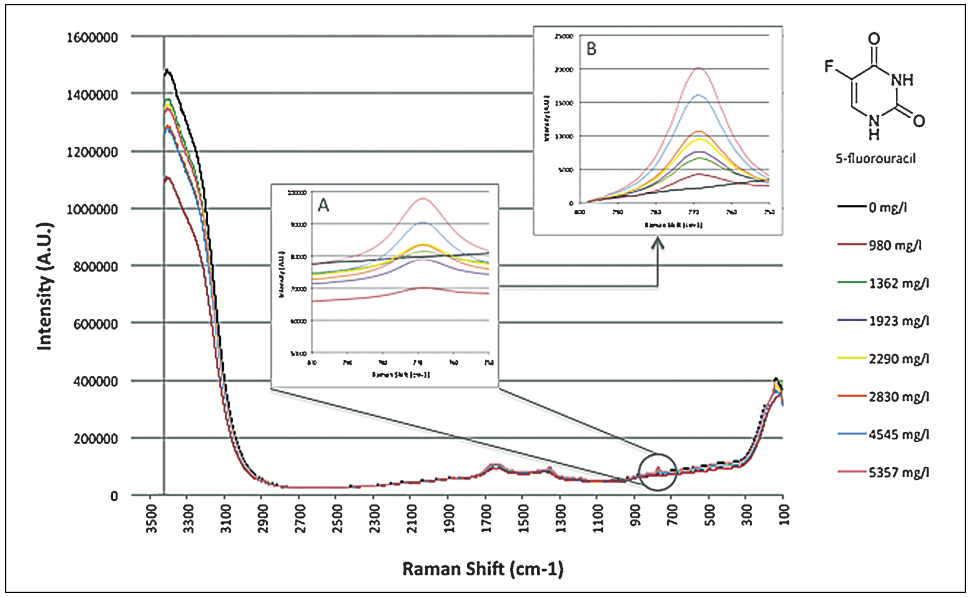

Fig. 1: Raman spectra of 5-FU diluted in NaCl 0.9% to final concentrations ranging from 0–5357 mg/l; (A) magnification of the region 750–800 cm^{-1}; (B) peak areas of the band at 769 cm^{-1} after baseline adjustment (A.U. = arbitrary units)

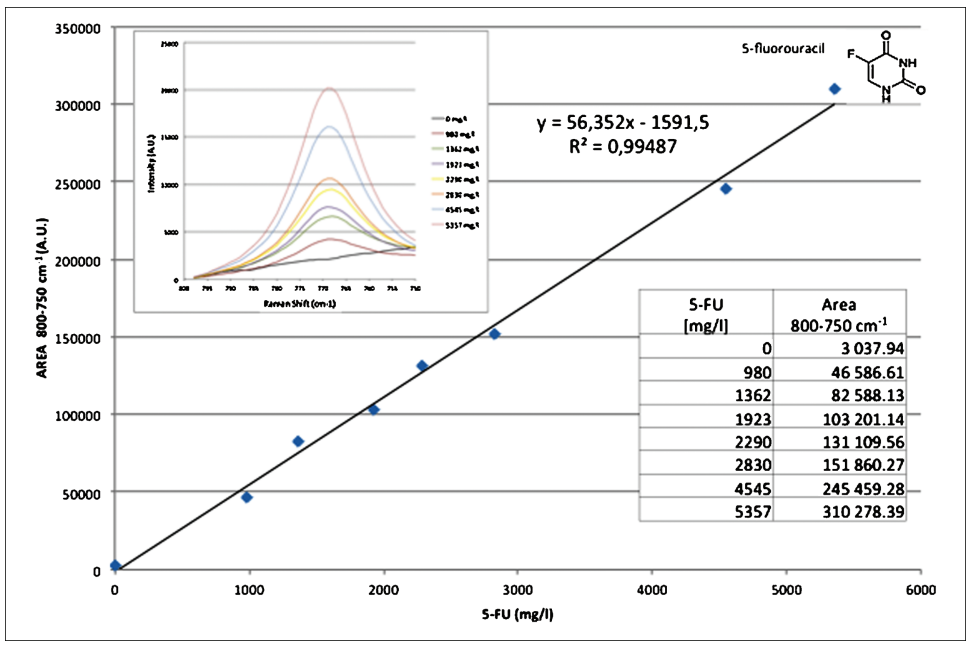

Fig. 2: Calibration curve of 5-FU; $r^2 > 0{,}99$

2. *Raman spectroscopy*

Raman spectra were obtained with a Raman setup RAMAN RXN1™ from Kaiser Optical Systems, Inc. (Ann Arbor, MI, USA). For Raman excitation an Invivtus™ NIR-laser, providing 785 nm with a laser power of about 450 mW was used. Spectra of 5-FU solutions were collected two times for 90 seconds and were analyzed by iC Raman™ 4.1 software from Kaiser Optical Systems, Inc. (Ann Arbor, MI, USA).

References

Airaksinen M, Otero MJ, Schmitt E, Cousins D (2006) Creation of a better medication safety culture in Europe: Building up safe medication practices. Viitattu 21.3. 2010.

Buckley K, Matousek P (2011) Recent advances in the application of transmission Raman spectroscopy to pharmaceutical analysis. J Pharm Biomed Anal 55: 645–652.

Farquharson S, Gift A, Shende C, Inscore F, Ordway B, Farquharson C, Murren J (2008) Surface-enhanced Raman spectral measurements of 5-fluorouracil in saliva. Molecules 13: 2608–2627.

Gordon KC, McGoverin CM (2010) Raman mapping of pharmaceuticals. Int J Pharm 417: 151–162.

Harz M, Kiehntopf M, Stöckel S, Rösch P, Deufel T, Popp J (2008) Analysis of single blood cells for CSF diagnostics via a combination of fluorescence staining and micro-Raman spectroscopy. Analyst 133: 1416–1423.

Harz M, Kiehntopf M, Stöckel S, Rösch P, Straube E, Deufel T, Popp J (2009) Direct analysis of clinical relevant single bacterial cells from cerebrospinal fluid during bacterial meningitis by means of micro-Raman spectroscopy. J Biophoton 2: 70–80.

März A, Mönch B, Rösch P, Kiehntopf M, Henkel T, Popp J (2011) Detection of thiopurine methyltransferase activity in lysed red blood cells by means of lab-on-a-chip surface enhanced Raman spectroscopy (LOC-SERS). Anal Bioanal Chem 400: 2755–2761.

Pérez-Cebrián M, Font-Noguera I, Doménech-Moral L, Bosó-Ribelles V, Romero-Boyero P, Poveda-Andrés JL (2011) Monitoring medication errors in personalised dispensing using the sentinel surveillance system method" Farmacia hospitalaria doi:10.1016/j.farma.2010.06.004.

Yuen C, Wei Zheng, Zhiwei Huang (2010) Low-level detection of anticancer drug in blood plasma using microwave-treated gold-polystyrene beads as surface-enhanced Raman scattering substrates. Biosensors Bioelectronics 26: 580–584.