The 8th International Electronic Conference on Medicinal Chemistry (ECMC 2022)
01–30 NOVEMBER 2022 | ONLINE

# Box-Behnken assisted HPLC development of simultaneous determination of doxorubicin and vorinostat encapsulated into polymeric nanoparticles

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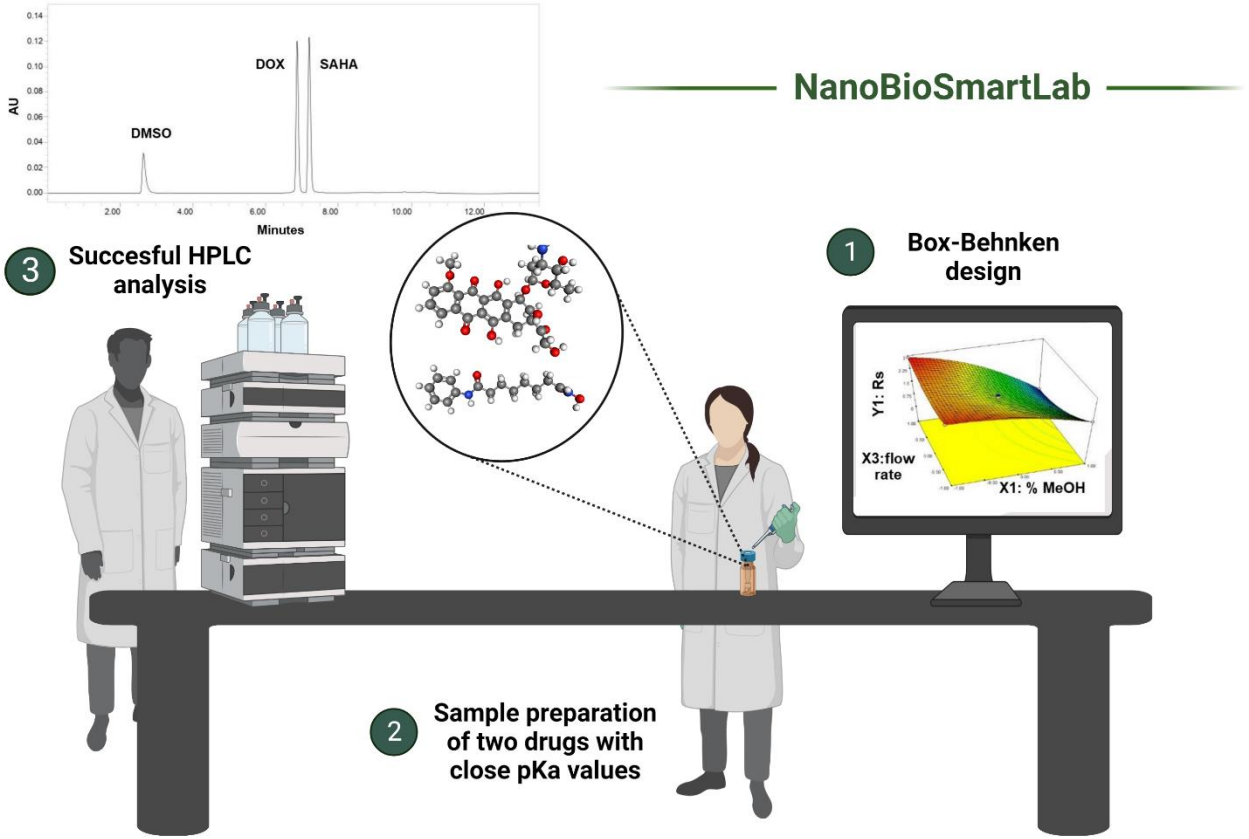
# Box-Behnken assisted HPLC development of simultaneous determination of doxorubicin and vorinostat encapsulated into polymeric nanoparticles

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**Abstract:** The objects of the present study are nanoparticles (NPs) based on a copolymer of lactic and glycolic acids (PLGA), loaded with the anticancer drug doxorubicin (DOX-NP) and histone deacetylase inhibitor vorinostat (SAHA-NP), developed for the breast cancer treatment. Drug encapsulation into PLGA matrix improve drug safety profile and allow to overcome multidrug resistance. In the current study, we developed a high-performance liquid chromatography method for the simultaneous determination of DOX-NP and SAHA-NP using Box-Behnken design, followed by the validation and NPs stability determination after sterilization treatment with γ-irradiation. The separation was performed using a Nucleodur C-18 Gravity column (250 mm × 4.6 mm × 5 µm). Samples were prepared by precipitating PLGA with dimethyl sulfoxide. Three independent variables were analyzed to determine the most optimal conditions: methanol concentration (0-20%), pH (2.5-4.5) and flow rate (0.8 -1.2 mL /min). We evaluated contributions of these variables to the peak resolution and the retention time of the last peak of the analyte using Box-Behnken design. Next, we simultaneously optimized all dependent variables and established their most optimal values using the desirability function. The optimized method was accurate, precise and linear in the range of 4.2–52.0 µg/mL for both drugs ($R^2$ = 0.9999 for vorinostat and $R^2$ = 0.9988 for doxorubicin). γ-irradiation at a dose of 25 kGy resulted in degradation of DOX-NP less than 95%, while the amount of SAHA-NP impurities was 88%. Thus, the developed method is suitable for simultaneous analysis of DOX-NP and SAHA-NP, including the analysis of impurities.

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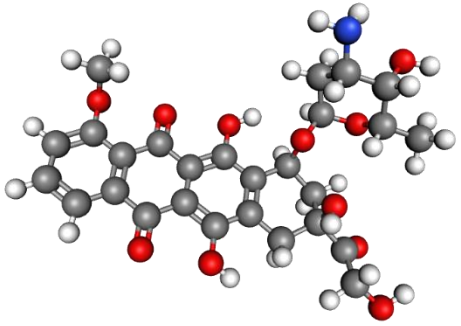
**Keywords:** Box-Behnken design; doxorubicin; nanoparticles; PLGA; validation; vorinostat

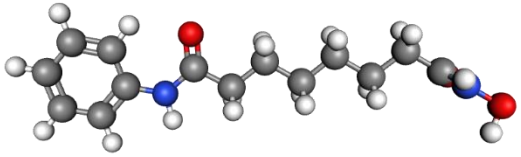
## Introduction

Doxorubicin (DOX) is a genotoxic agent exhibiting antimitotic and antiproliferative effects by means of interaction with DNA. The empirical formula of DOX is $C_{27}H_{30}ClNO_{11}$ and its molecular weight is 580.0 g/mol (**Fig. A**). Vorinostat (suberoylanilide hydroxamic acid, SAHA), a histone deacetylase inhibitor (HDACi), is an FDA-approved drug for cutaneous T-cell lymphoma. It is a linear hydroxamic acid compound, with an empirical formula of $C_{14}H_{20}N_2O_3$ and a molecular weight of 264.3 g/mol (**Fig. B**). Both analytes are bases with pKa values of 8.2 and 9.2 for DOX and SAHA, respectively.

**A**



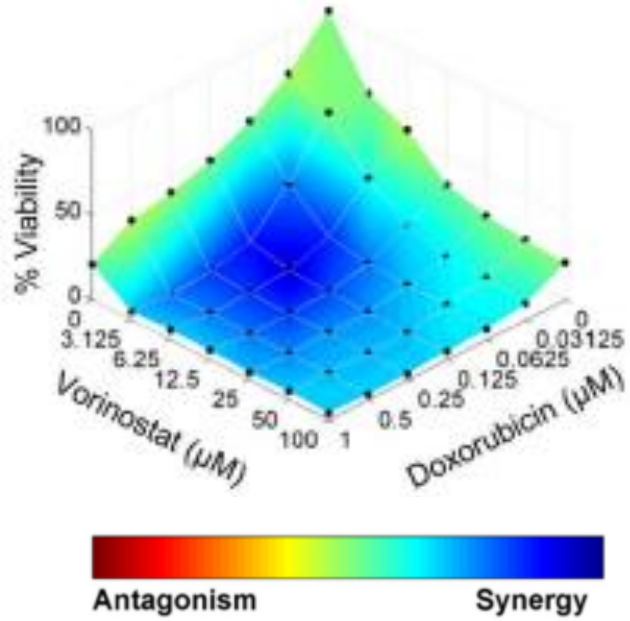
**B**



Molecular structures of **(A)** doxorubicin and **(B)** SAHA.
Created with MolView https://molview.org/

# Introduction

While histone deacetylase inhibitors, such as SAHA, demonstrate significant effect against hematological cancers, its application for solid tumors treatment is limited. However, there is strong evidence that combinatorial administration of SAHA and genotoxic agents (e.g., DOX) enhance the antitumoral action of both drugs against tumors. SAHA influence on chromatin relaxation can increase the cytotoxic effect of DOX [1]. In phase I trial Munster and coauthors reported DOX and SAHA can be safely combined and are promising in treatment of solid tumors [2]. These founding give a promising approach of solid tumors' combinational therapy with DOX and SAHA.



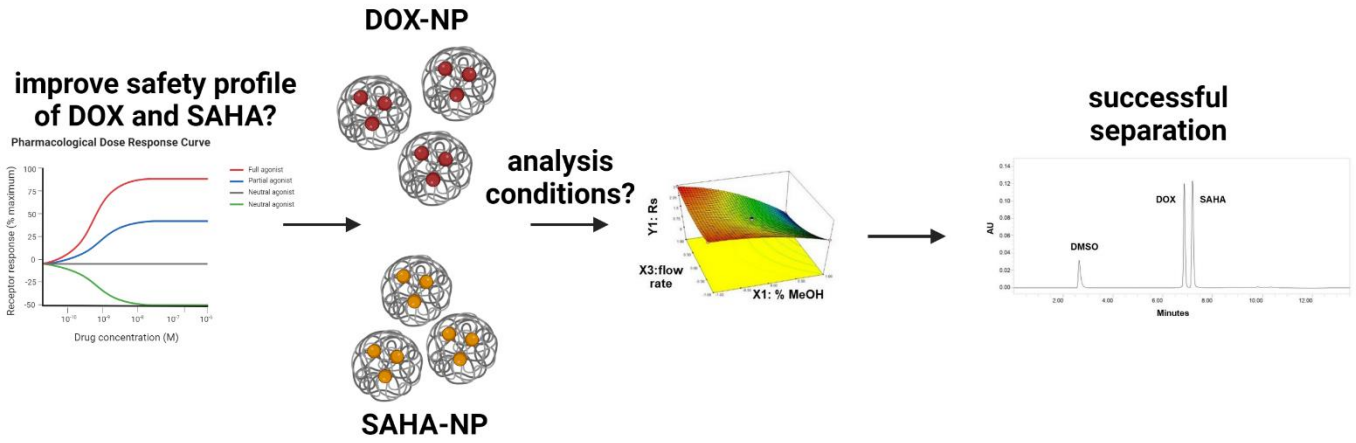

Synergistic effects of HDACi and doxorubicin on MDAMB-468 [1].


*[1] Hii L. W. et al. Histone deacetylase (HDAC) inhibitors and doxorubicin combinations target both breast cancer stem cells and non-stem breast cancer cells simultaneously //Breast cancer research and treatment. – 2020. – Т. 179. – №. 3. – С. 615-629.*

*[2] Munster P. N. et al. Phase I trial of vorinostat and doxorubicin in solid tumours: histone deacetylase 2 expression as a predictive marker //British journal of cancer. – 2009. – Т. 101. – №. 7. – С. 1044-1050.*

# Introduction

In current research we designed DOX-loaded (DOX-NP) and SAHA-loaded (SAHA-NP) nanoparticles based on copolymer of lactic and glycolic acid (PLGA). PLGA is biocompatible and biodegradable polymer that is extensively used for medical applications. The encapsulation of drugs into nanoformulations improve the safety profile of drugs and allow overcoming drug resistance, which mainly limit the efficacy of both drugs. The present study demonstrates the development, optimization with Box-Behnken design, and validation of a novel HPLC-UV method for simultaneous quantitation of DOX and SAHA in polymeric nanoformulations.



# Results and discussion

We applied Box-Behnken design to develop novel HPLC method. We used three variables including methanol concentration (%), pH, and flow rate of mobile phase to explore their contribution to resolution (Rs) and retention time of the last analyte peak (RT SAHA).

List of dependent and independent variables in Box-Behnken design for HPLC optimization.

| Independent variables | | | | Dependent variables |
|---|---|---|---|---|
| Factors | | Levels | | Responses |
| | -1 | 0 | +1 | |
| Methanol concentration, % (X1) | 0 | 10 | 20 | Rs (Y1) |
| Flow rate, mL/min (X2) | 0.8 | 1 | 1.2 | RT SAHA, min (Y2) |
| pH (X3) | 2.5 | 3.5 | 4.5 | |

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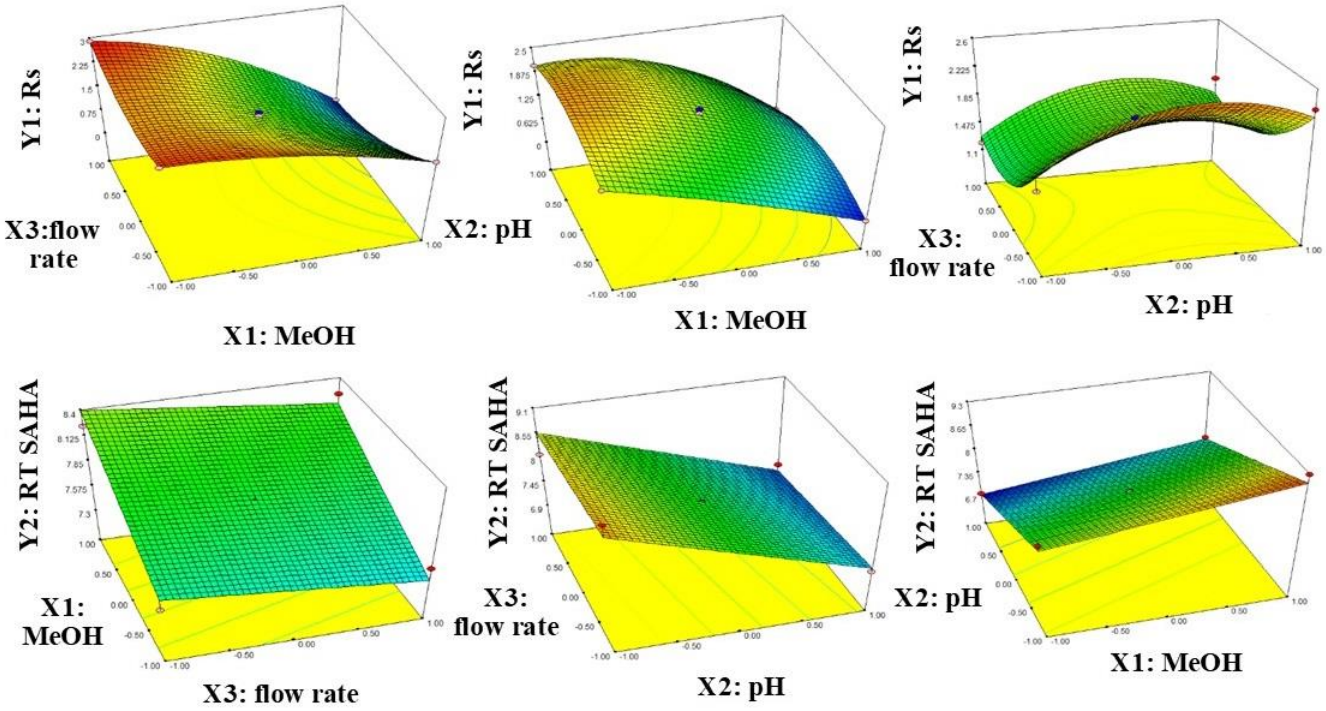
# Results and discussion




Box-Behnken design clearly indicate that the Rs and RT SAHA values are strongly affected by the MeOH percentage (*X1*), flow rate (*X2*), and pH value (*X3*).

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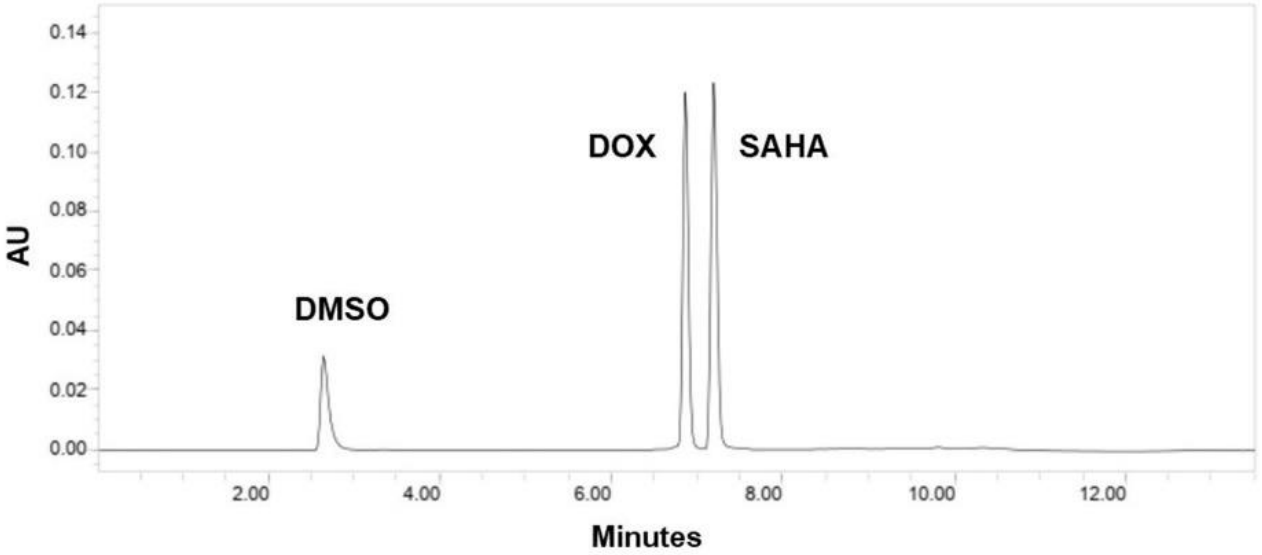
## Results and discussion

We screened all the conditions with Design Expert software to determine the optimized conditions, which are corresponded to the maximum desirability value. A maximum desirability of **0.954** corresponded to conditions of **0% MeOH**, **flow rate = 1.2 mL/min** and **pH = 3.9**. The observed values of responses showed good **resolution (2.6 ± 0.02)** and suitable **retention time of SAHA (7.2 ± 0.02 min)**.




The chromatogram of standard mixture solution containing 25 µg/mL of DOX and 25 µg/mL of SAHA obtained under the optimized conditions.

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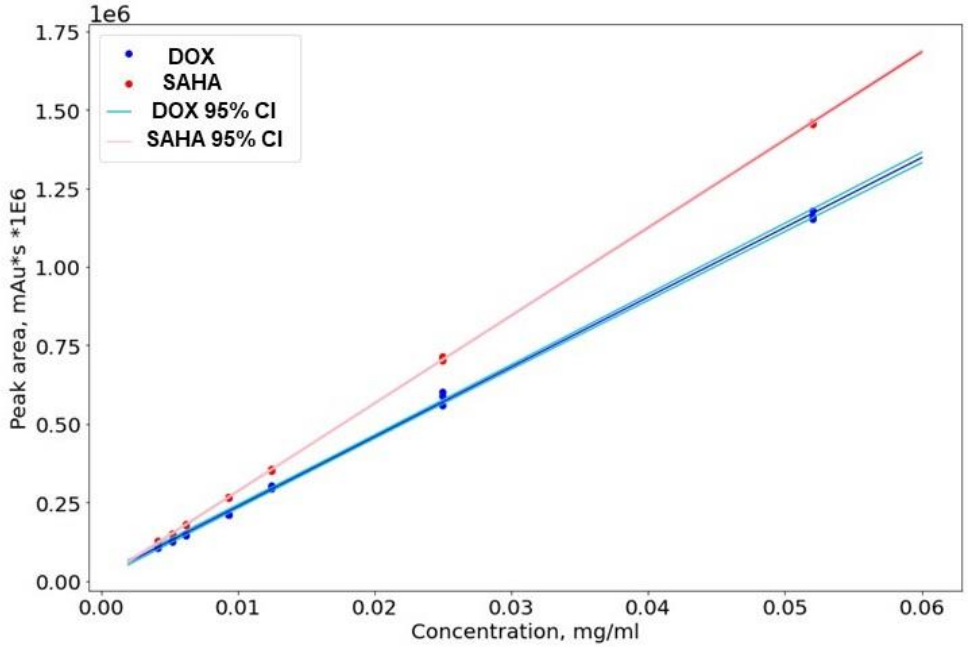
# Results and discussion

We validated the developed method according to ICH guidance. The method was linear within the concentration range of 4.2-52.0 µg/mL for both drugs with LOD and LOQ values of 3.5 and 10.7 µg/mL for DOX and of 2.5 and 7.7 µg/mL for SAHA, respectively. The method was precise and accurate over the concentration range of analysis.




Plot of a calibration curve (OLS fitting). The band indicates the 95% confidence interval.

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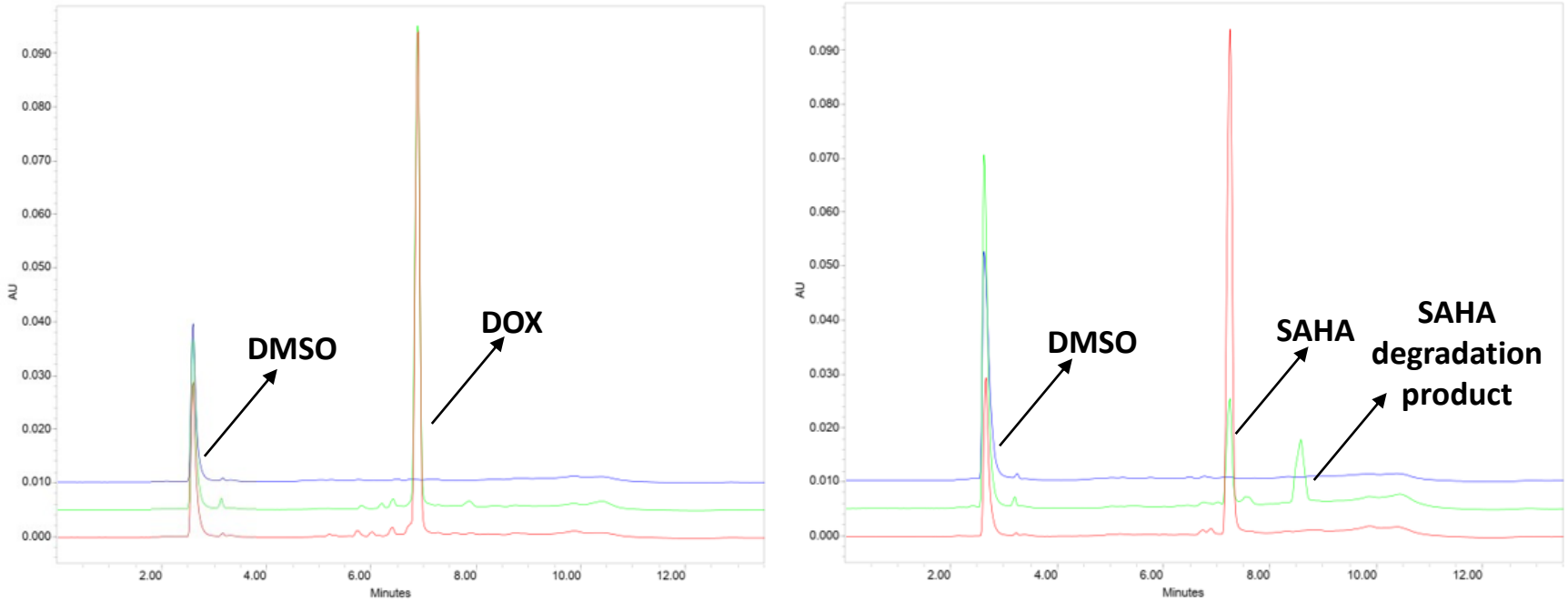
# Results and discussion

γ-irradiation resulted in increase of DOX impurities from 3.7% to 4.6%, however, it was below 5%. The amount of SAHA degradation products was 88%, the peak being well distinguished in chromatogram. Our results indicate instability of SAHA-NP after irradiation at a dose of 25 kGy.




HPLC chromatograms of **A)** DOX-NP (red), DOX-NP after irradiation (green), blank NPs after irradiation (blue); **B)** SAHA-NP (red), SAHA-NP after irradiation (green) and blank NPs after irradiation (blue).

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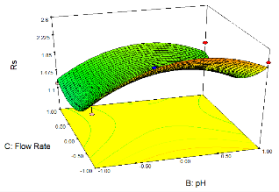
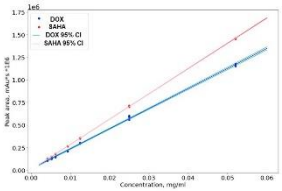
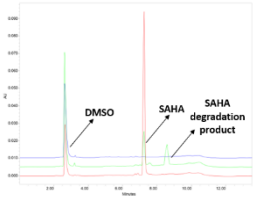
# Conclusions



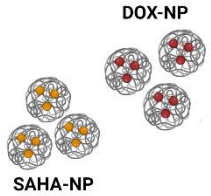

Surface methodology based on BBD performed successfully simultaneous optimization of methanol concentration, flow rate and pH value.




According to validation results the developed method is precise, accurate, and provides reproducible quantitative analysis.




After sterilization of SAHA-NP via gamma-irradiation 88% of the substance degraded.




The method was suitable for simultaneous drug loading determination of two individual PLGA nanoformulations, providing simple and fast HPLC analysis.