J Chromatogr Sep Tech 2017, 8:4(Suppl)
DOI: 10.4172/2157-7064-C1-029

Kinetics of trapping of O-donor compounds by silylene

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This work was part of series of experiments using UV laser at 193 nm to generate SiH_2 (at 343 K) and MeSiH (298 K) in the presence of O-donor compounds. Phenylsilane (PhSiH3) was employed as precursors to monitor and study the reaction of SiH_2. The motivation was to detect and monitor the products of reactions. A method for the determination of rate constant for SiH_2 reactions relative to the rate constant of $PhSiH_3$ was formulated. This was used to determine some relative rates of insertion reactions of silylene (SiH_2) with MeOMe, MeOH, and H_2O. Two chromatographic systems were used for analysis, both of which were equipped with gas sampling valves. The gas sampling loop was heated to 343 K by means of electrothermal heating tape to minimize problems of adsorption. In addition several types of columns were employed for analytical purposes, the packing material and operating conditions of which were dependent on the reaction under investigation. Chromatography was used as an analytical method to determine the composition of the reaction product mixture. An Oxford KX2 excimer laser operating on the ArF laser transition at 193 nm was used as the source of photolysis radiation. The insertion reaction of SiH_2 into O-donors showed a rate enhancement effect of methyl-substitution in the substrate, although no products such as $H_2MeSiOMe$ were identified.

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Development and validation of analytical methods based on RP-HPLC: Quantifying HER1 extracellular domain in culture supernatant and peptide mapping of a monoclonal antibody

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Techniques based on high-resolution liquid chromatography are currently widely used to quantify recombinant proteins from culture supernatants, as well as their characterization. Such assays can be easily and rapidly developed, in case of reverse phase chromatography. In this study, we describe the development and validation of an analytical technique for quantifying HER1 extracellular domain (HER1 ECD) in bioreactor supernatant using reversed-phase chromatography with a C8 column. On the other hand, we validated the methodology for the peptide mapping of monoclonal antibody using C4 column. For both study cases, the proteins were analyzed by monitoring the absorbance of the sample at 214 nm. The resulting analytical methodology was found to provide precise and accurate results for a wide range of concentrations (10–120 µg/mL) of HER1 ECD. The accuracy of the method varied from 86 to 109%, while the repeatability and the day-to-day intermediate precision were less than 7.25 and 7.85%, respectively. In the case of peptide mapping of mAb, the methodology provides a range of 35-40 well resolved peaks. As a criterion is set, RT≤0.5 min and the % peak height relative to the reference material must be 70% to 130%. These methodologies constitute a useful tool that can be applied during the production of the HER1 ECD vaccine and in the identification of modifications on the primary structure of the mAb due to changes in biomanufacturing process.

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