

**Electrophoretic and chromatographic profile of a highly water-soluble fullerene derivative
Analysis of purity and ascertainment in urine following IP injection to rodents**

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Electrophoretic and chromatographic behavior of a highly water-soluble Recently, it has been shown that some fullerene 1 derivatives exhibit considerable biological activity. However, in order to evaluate the in vivo behavior of this family of new compounds, it is essential to investigate their metabolic fate after administration, together with their potential toxicity. For this purpose, it is necessary to use very pure and well-defined chemical compounds. Several analytical methods like TLC, NMR, MS, IR and UV-VISIBLE spectroscopy have been used to identify fullerenes and their derivatives. Surprisingly, most authors neglect to check the purity of the fullerene derivatives they use in their studies using effective methods of separation. In the same way, only a few studies were performed on the in vivo toxicity and metabolic fate of this family of new compounds, which is probably due to the need for sensitive analytical methods. Although high-performance liquid chromatographic (HPLC) methods are used to analyze and purify fullerenes, only one paper describes the use of this technology for analyzing the hydrophobicity of water-soluble C60 derivatives. Capillary electrophoresis (CE) techniques have also been used to separate fullerenes, but to the best of our knowledge these methods have never been used to analyze their derivatives. Among the variety of C60 derivatives synthesized until now, two are prone to be used for medical purpose. The first is the highly water-soluble dendro[60]fullerene (DF), which has been shown to be active against HIV-1 in vitro. The second compound, a trisdicarboxymethanofullerene-C60 (TDCF), is also water-soluble and exhibits interesting neuroprotective activity. In order to investigate the purity of these compounds as well as their possible metabolism in vivo, it is necessary to develop sensitive analytical methods for analyzing them. We report here the systematic investigation of the capillary zone electrophoresis (CZE) and the reversed phase liquid chromatographic (RP- LC) behavior of DF, taken as a model of water-soluble C60 derivative. Two parameters are of the utmost importance as to their effect on the migration time and separation efficiency of DF by CZE: pH and ionic strength whose actions are opposite. The chromatographic separation of this highly water-soluble C60 derivative obeys the general rules of stationary phase and mobile phase selection for controlling the separation of usual acidic compounds. As a consequence, CL- ESI-MS has been applied to estimate the purity of a sample of DF. We also used RP-HPLC to analyze a sample of a regio-isomer of TDCF. The results obtained clearly show that is necessary to use a powerful method of separation to control the syntheses of fullerene derivatives as well as to check their purity

before considering their use for medical purposes. We also report the use of HPLC-ESI-MS to determine DF in urine of rodent after an intraperitoneal single dose administration.