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Analysis of steroids in human urine by on-line coupled liquid chromatography-gas chromatography-mass spectrometry (LC-GC-MS) and liquid chromatography-gas chromatography-combustion-isotope ratio mass spectrometry (LC-GC-C-IRMS) using the through oven transfer adsorption desorption interface

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Anti-doping laboratories accredited by the WADA (World Anti-Doping Agency) must have available methods capable of detecting synthetic steroids at concentrations below 10 ng mL⁻¹. Most accredited laboratories use gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS) to discriminate between natural and synthetic steroids, and gas chromatography-mass spectrometry GC-MS to identify the analyte peak. The method involves laborious sample preparation, including hydrolysis, liquid-liquid extraction and acetylation. A further cleaning step of the derivatized steroids is usually carried out by liquid chromatography (LC) to ensure the purity of the steroid. In the analytical methods presented, the Through Oven Transfer Adsorption Desorption (TOTAD) interface was used to couple the last clean up stage of LC with GC-IRMS or GC-MS to provide on-line coupled LC-GC-IRMS and LC-GC-MS analytical methods. The methods developed were used to analyse 11-hydroxyandrosterone, 11-ketoetiocholanolone, epitestosterone, testosterone, etiocholanolone, androsterone, 5 α Adiol, 5 β Adiol and pregnadiol, as well as Boldenone and its principal metabolite. The volumes transferred from the LC to GC range from 700 to 2200 μ L, while acetonitrile/water was used as the LC mobile phase. The TOTAD interface eliminates the solvent but retains steroids that are introduced in the GC column. Good sensitivity is achieved, detection limits being below 5 ng mL⁻¹ in all cases, as the whole LC fraction containing the analytes is transferred from LC to GC. The relative standard deviation (RSD) of the absolute peak areas is below 20% for MS detection, and the RSD of the $\delta^{13}\text{C}$ is about 1.1%, both of which can be considered very good since they represent the variability in the whole process, including sample preparation. While developed for antidoping purposes, the LC-GC-MS methodology can be applied for other analytical goals.

Biography

Jesus Villen worked on Large Volume Injection (LVI) for his PhD thesis, which was completed in 1993. His Postdoctoral research was into the development of the TOTAD interface, and its uses for LVI in GC as well as for on-line LC-GC coupling. The TOTAD interface was first patented 1998 and since then, the research group, "Analysis by on-line LC-GC" and headed by him, has been dedicated to improving the TOTAD as well as to developing LVI and LC-GC analytical methods using this interface. He is the author of 10 patents, four of which have been extended to the USA, Europe and other countries, as well as of about 35 papers published in journals included in the first quartile. He has directed numerous national and international research projects. Besides his research, he teaches chemistry to future forestry engineers.

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