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From the sixteenth to the eighteenth centuries, the Spanish and Portuguese Inquisitions conducted a number of trials against individuals accused by members of their communities of being of the other gender – men accused of being women and women accused of being men – or even hermaphrodites. Using new inquisitorial sources, this study examines the complexities revolving around transgenderism and the construction of gender identity in the early modern Iberian World. It throws light upon the manner in which the Inquisition, medical practitioners and the wider society in Spain and Portugal responded to transgenderism and on the self-perception of individuals whose behaviour, whether consciously or unconsciously, flouted these social and sexual conventions.