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ORIGINAL ARTICLE

Development of efficient SPE–TLC method and evaluation of biological interactions of contraceptives with progesterone receptors

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Protein bindings

Abstract TLC–SPE methodologies were developed to ascertain biological interactions of norethindrone acetate and dydrogesterone contraceptives with plasma progesterone receptor proteins. TLC solvent system for plain and Cu(II) impregnated silica gel plates was *n*-hexane-*n*-butanol (90:10, v/v), which took 20 min to run up to 10.0 cm. The best separation was on Cu(II) impregnated plates due to maximum difference in R_f values and compact spots. The optimized SPE conditions were pH 2.0 and 3.0 of phosphate buffer (50 mM) for norethindrone acetate and dydrogesterone, respectively. The flow rate of plasma and eluting solvent (methanol) through C_{18} cartridge was 0.10 mL/min. The interactions of these contraceptives with progesterone receptor proteins were analysed by TLC–SPE results, which were supported by modelling using PyMOL and Autodoc4 softwares. The dydrogesterone was found to be bound strongly than norethindrone acetate. Attempts have been made to discuss the drugs' interactions at chemo-supramolecular level.

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1. Introduction

Nowadays, Asian and African countries are under great pressure due to geometrical growth of population, effecting the economy and ecosystem of the whole world. The control of this alarming problem is essential and an urgent need of today. The population control is being achieved through various contraceptive approaches. During the last four decades, many contraceptive methods have been developed and used; and among them oral dosages are adopted globally (Mansour 2005; Coelingh Bennink et al., 2003). Most commonly used oral contraceptive drugs are based on estrogens and progesterone hormones. Among many, norethindrone acetate

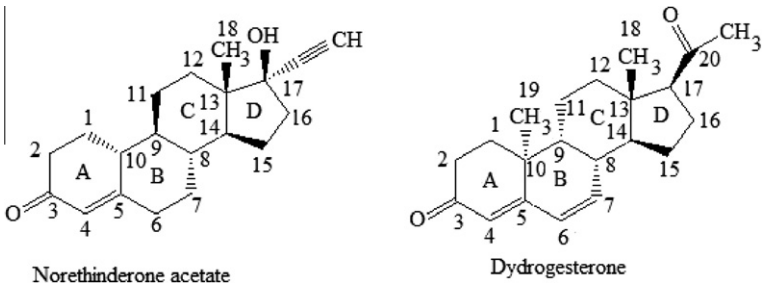

Figure 1 Chemical structures of norethindrone acetate and dydrogesterone contraceptives.

and dydrogesterone Figure 1 are the most commonly used progestogenic components in hormone replacement therapy (HRT); with about 80% in the market (Coelingh Bennink et al., 2003; Li et al., 2005). These progestogens have been used world widely as oral contraceptive preparations for many years with sales of about billions of US $ per year (Willard et al., 2003). In spite of their crucial role in birth control, these medications result into some side and toxic effects, that require the exploration of the action of mechanism for further improvement in their molecular structures. The interactions of these drugs with protein are the main core of the mechanism of action. Literature indicates that these drugs bind with progesterone receptor proteins (Madauss et al., 2004) but the binding profile and pattern is not well known. These types of studies require the efficient analytical methods and modelling software. A thorough search of literature indicates only few analytical methods for monitoring these drugs. Among them, chromatography is an ideal technique and HPLC (Matlin et al.,1983; Gonzalo-Lumbreras and Izquierdo-Hornillos, 2000; Sundaresan et al., 2006) and TLC (Simard and Lodge, 1970) have been used for this purpose. Of course, HPLC is a more advanced and popular modality, however, thin layer chromatography (TLC) has its unique feature of inexpensiveness, parallel chromatographic profiles of unknowns and standards and ease of operation (Stahl1969; Fried and Sharma, 1991; Fried and Sharma, 1996). Therefore, TLC method development is the real demand for these drugs due to explosion of population in developing countries, where TLC suits well as an ideal technique (inexpensive).The monitoring of analytes in plasma needs sample preparation before loading onto TLC. It has been observed that about 80 percent chromatographers are using solid phase extraction (SPE) as the versatile method of sample preparation for plasma (Ali et al., 2008). It is interesting to observe that no method is available for monitoring these drugs in plasma by using SPE–TLC combination, that we found ideal for third world countries. In view of these facts, attempts have been made to develop inexpensive, fast, selective and reproducible SPE–TLC methods for analyses of the reported contraceptives in human plasma. Based on the results obtained and modelling, efforts were made to explain the interactions of these drugs with progesterone receptor protein for further research. The results of these findings are discussed herein.

2. Experimental

2.1. Chemicals, reagents and instruments

Fresh frozen human plasma (Mfg. Licence No. 504) was purchased from Rotary Blood bank, New Delhi India. Methanol, *n*-hexane, *n*-butanol and silica gel G, disodium hydrogen phosphate and *o*-phosphoric acids were purchased from Merck, India. Ferric chloride, sulphuric acid and glacial acetic acids were obtained from Qualigens, India. Standard solutions ($0.10\ mg\ mL^{-1}$) of these drugs were prepared in methanol. Norethindrone acetate and dydrogesterone were detected on TLC plates by developing a new reagent. The reagent was prepared by dissolving 500 mg ferric chloride in a mixture of sulphuric acid (20 mL) and glacial acetic acid (10 mL) followed by water dilution up to 50 mL. Purified water was prepared using a Millipore Milli-Q (Bedford, MA USA) water purification system., Sep-Pak C_{18} 1 mL barrel size cartridges containing 50 mg of sorbent (particle size 55–105 μm and pore size 125 Å) were purchased from Waters USA (Cat.No. WAT054955). pH meter of Control Dynamics (model APX 175 E/C), spectrophotometer of Perkin Elmer (model EZ201), solid phase extraction unit of VARIAN and centrifuge (model C854/49/06) of Remi were used. PyMOL visualization tool and Autodoc4 software were used for modelling purposes of drugs binding with progesterone receptor.

2.2. Extraction of drugs from commercial tablets

Norethindrone acetate and dydrogesterone were extracted from commercially available tablets. Norethindrone acetate was extracted from five regestrone tablets (batch No. R67B05D) of Novartis India Limited, Mahad, (Maharashtra), India. Similarly, dydrogesterone was extracted from five Duphastone formulation (batch No. L7301) of Solvay Pharma India Limited, Mumbai, India. Five tablets of each drug were weighed and crushed to powder separately, respectively. The powdered tablets were extracted with methanol (100 mL), separately, by heating at 80 °C. The drug mixture was centrifuged and supernatant was separated. The residue was extracted two more times with the same amount of methanol separately. All methanol fractions were combined together to get 300 mL. This methanol was evaporated under vacuum on water bath to 15 mL, which was allowed to crystallize in freeze at 10 °C. The mother liquor was decanted and the crystals were washed with a little amount of *n*-hexane. The purity of the drugs was ascertained by melting point, UV and IR spectra.

2.3. Preparation of TLC plates

TLC plates ($10\ cm \times 15\ cm \times 0.5\ mm$) were prepared in the laboratory by spreading slurry of silica gel G (50 g) in 100 mL Millipore water. These plates were dried overnight in an oven at 80 °C. For impregnated TLC plates, silica gel slurry was prepared with Millipore water containing 0.10 g each of